SODIUM, POTASSIUM AND CHLORIDE NUTRITION OF THE LACTATING DAIRY COW: INFLUENCE OF DIETARY CATION-ANION INTERRELATIONSHIPS ON ACID-BASE STATUS AND LACTATIONAL PERFORMANCE

BY

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To my wife, who gave me a reason;
to my father, who gave me wisdom; and,
to my late brother, who gave me an example.

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LIST OF ABBREVIATIONS

Excluding abbreviations of common weights and measures, the following are used within the dissertation. Terms may or may not be accompanied with long form descriptions in the text.

ADF = Acid detergent fiber
ANGAP = meq [(Na + K) - (Cl + HCO_3^-)] in whole blood or plasma
ANOVA = Analysis of variance
ARC = Agricultural Research Council
BE = Blood base excess
BW = Body weight
BWG = Body weight gain
Ca = Calcium
Ca^{++} = Calcium ion(s)
$CaCl_2$ = Calcium chloride
$CaCO_3$ = Calcium carbonate
CAD = Cation-anion difference expressed as meq (Na + K - Cl)/100 g diet DM
CCD = Central composite design
CF = Crude fiber
Cl = Chloride
Cl^- = Chloride ion(s)
Co = Cobalt
CO_2 = Carbon dioxide
CP = Crude protein
Cr_2O_3 = Chromic oxide
Cu = Copper
d = Day(s)
df = Degrees of freedom
DHIA = Dairy Herd Improvement Association
DIM = Days in milk or stage of lactation
DM = Dry matter
DMI = Dry matter intake
DUA = Dietary undetermined anion
ECF = Extracellular fluid
FCM = Fat-corrected milk
Fe = Iron
GLM = General linear models
h = Hour(s)
H^+ = Hydrogen ion(s)
$[H^+]$ = Hydrogen ion concentration
HCO_3^- = Bicarbonate ion(s)
HK:HCl = High K, high Cl
HK:LCl = High K, low Cl
H_2O = Water

$HPO_4^=$ = Dibasic phosphate
$H_2PO_4^-$ = Monobasic phosphate
I = Iodine
K = Potassium
K^+ = Potassium ion(s)
KCl = Potassium chloride
K_2CO_3 = Potassium carbonate
$K_2Cr_2O_7$ = Potassium dichromate
HCl = Hydrochloric acid
LK:HCl = Low K, high Cl
LK:LCl = Low K, low Cl
LSMS = Least squares means
meq = milliequivalent(s)
MF = Milk fat
Mg = Magnesium
$MgCl_2$ = Magnesium chloride
MgO = Magnesium oxide
Mg_2SO_4 = Magnesium sulfate
MLCa = Milk Ca
MLCl = Milk Cl
MLK = Milk K
MLMg = Milk Mg
MLNa = Milk Na
Mn = Manganese
MP = Milk protein
MY = Milk yield
Na = Sodium
Na^+ = Sodium ion(s)
NaCl = Sodium chloride
$NaHCO_3$ = Sodium bicarbonate
Na_2HPO_4 = Sodium phosphate
NDF = Acid detergent fiber
NE_L = Net energy of lactation
NH_3 = Ammonia
NH_4^+ = Ammonium ion(s)
NH_4Cl = Ammonium chloride
$(NH_4)_2SO_4$ = Ammonium sulfate
NRC = National Research Council
OH^- = Hydroxide ion
P = Phosphorous or probability. Probability is associated with a coefficient between 0 and 1 and the < and > symbols.
pH = Negative logarithm of $[H^+]$
PCa = Plasma Calcium
PCl = Plasma Cl
pCO_2 = partial pressure of CO_2
PK = Plasma K
PMg = Plasma Mg
PNa = Plasma Na
R^2 = Coefficient of determination
RBS = Red blood cell
S = Sulfur

SAS = Statistical Analysis System
Se = Selenium
SEM = Standard error of the mean
SID = Strong ion difference
SiO_2 = Silicon dioxide (washed sand)
TMR = Total mixed ration
UIP = Undegradable intake protein
USDA = United States Department of Agriculture
WBCa = Whole Blood Ca
WBCl = Whole Blood Cl
WBK = Whole Blood K
WBMg = Whole Blood Mg
WBNa = Whole Blood Na
WK = Week(s)
Yr = Year(s)
Zn = Zinc
Zn_2SO_4 = Zinc sulfate

Abstract of Dissertation Presented to the Graduate School
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By

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Objectives were to determine the influence of dietary sodium (Na), potassium (K), and chloride (Cl) on acid-base status and lactational performance of dairy cattle. Experiment one was conducted to study individual and interrelated effects of varying concentrations of Na, K, Cl and cation-anion difference (CAD; milliequivalents (Na + K - Cl)/ 100 grams diet). Five concentrations of Na (.31 to .85%), K (.86 to 1.96%), and Cl (.32 to 1.15%) in a central composite design were fed to 48 midlactation cows. Dietary CAD ranged from +12 to +62. Dietary concentrations of Na, K and Cl influenced lactational performance. Optimal concentrations were above current recommendations but were interdependent. Dietary Na spared dietary K (and vice versa). Increasing dietary Cl was deleterious unless accompanied by an increase in dietary Na and/or K. Optimum CAD was between +23 and +50.

Experiment two was conducted to evaluate source of Na, K and Cl (sodium bicarbonate, sodium chloride and potassium chloride). Seven

diets with mixtures of these salts (1% of diet dry matter) and one control diet were fed to 36 midlactation cows. Treatments were defined according to a simplex-centroid mixtures design. None of the mixtures had a significant influence.

Data from these experiments were combined with eight others for empirical modeling. Objectives were to identify and quantify effects of macromineral interrelationships and CAD on lactational performance. Performance was optimal at .58% Na, .40% Mg and +38 CAD. Consistent interrelationships between Na and K, Na and calcium (Ca), K and Ca, and K and Cl were found.

A final experiment was conducted to determine physiological responses to K x Cl interaction. Hourly and daily blood and urine samples were collected. Cows were assigned to a 4 x 4 Latin square and fed diets with low and high concentrations of K factored with low and high concentrations of Cl (1.1 versus 1.8% K and .43 versus .91% Cl). The data suggested a physiological mechanism for K x Cl interaction was present. The diet with high Cl and low K did not contain sufficient cation to accompany excess Cl into the urine. Chloride was retained and subclinical hyperchloremic metabolic acidosis developed.

The need to consider dietary macromineral interrelationships and CAD for lactating dairy cattle was established. Results are directly applicable to the dairy industry and should provide economic benefit.

CHAPTER 1
INTRODUCTION

The first general review of mineral metabolism in this country was published in 1922 (Mattill and Mattill, 1922). Since then researchers have identified as many as 36 elements that are nutritionally important to domestic animals (NRC, 1980). Volumes have been written about the singular effects of each element, but comparatively little is known about their interrelated effects. Twenty years ago Jacobson et al. (1972) suggested that mineral interrelationships are the least understood and most promising area of mineral nutrition of dairy cattle.

With much foresight, Shohl (1939), writing about limitations of knowledge in mineral metabolism, noted that when mineral functions are described as the action of single elements, a degree of simplicity is assumed erroneously. He further suggested that the relations of elements to one another, to water, to acid-base equilibrium, and to body functions are so complicated that such a simplistic scheme no longer was adequate. Over 20 years ago Paquay et al. (1968) recognized the importance of mineral interrelationships and suggested that the metabolism of ration constituents depends on the other dietary constituents nearly as much as on their own intake and sometimes even more. In spite of these early claims, current mineral requirement guidelines (ARC, 1980; NRC, 1989) do not adequately account for potential dietary interrelationships.

Designing diets to meet the nutritional needs of the high producing dairy cow is a challenge. As a result of improved genetics, nutrition, health and other management practices, milk yields per cow have doubled in less than 30 years (USDA, 1990). Yet the efficiency of milk production must continue to improve if the dairy industry is to maintain or enhance its role as an important producer of food (NRC, 1989). As the genetic potential of our dairy cattle population continues to increase, designing diets with the optimum concentration of minerals becomes even more critical. Concentrations of the monovalent macrominerals, sodium (Na), potassium (K) and chloride (Cl), are particularly important to lactating cows. Interrelated effects among dietary concentrations of these three macrominerals have been suspected for some time (Shohl, 1939), but few theories have been proposed to explain how they affect lactational performance of the dairy cow.

Shohl and Sato (1923) were first to propose that mineral interrelationships were related to acid-base status. Shohl (1939) included a chapter in his book entitled Cation-Anion Relationships and proposed that maintenance of normal acid-base equilibrium required excretion of excess dietary cations and anions. He hypothesized that consumption of either excess mineral cations relative to anions or excess anions relative to cations resulted in acid-base disturbances.

Once animal nutritionists began to test this hypothesis, mineral interrelationships were found to affect numerous metabolic processes. Leach (1979) and Mongin (1980) reviewed related literature and concluded that mineral interrelationships had profound influences. They theorized that for an animal to maintain its acid-base homeostasis, input and

output of acidity had to be maintained. It was shown that net acid intake was related to the difference between dietary cations and anions. The monovalent macromineral ions, Na, K and Cl were the most important elements in the expression (Mongin, 1980).

Mongin (1980) was one of the first to propose a three-way interrelationship among dietary Na, K and Cl. He proposed that the sum of Na plus K minus Cl (in meq per 100 g diet DM) could be used to predict net acid intake. This sum commonly has been referred to as the dietary cation-anion balance (Tucker et al., 1988a) or dietary electrolyte balance (West et al., 1990) but Sanchez and Beede (1991) coined the term "cation-anion difference" to represent the mathematical calculation used more precisely and to avoid the erroneous connotation that mineral cations truly are balanced with mineral anions in the diet.

Effects of cation-anion difference (CAD) in poultry have been studied extensively. Feeding low (i.e., below +20) dietary CAD, particularly from excessive mineral anions, has disturbed acid-base status, growth rate and eggshell mineralization, and has increased the incidence of tibial dyschondroplasia in poultry (Austic, 1988). Weanling pig growth also has been reduced by feeding diets with relatively low CAD (Yen et al., 1981).

Tucker et al. (1988a) were first to evaluate this concept with lactating dairy cattle and suggested that there may be an optimal CAD for achieving maximal intake and milk production. They compared diets formulated with -10, 0, +10 and +20 meq/100 g CAD and reported that a diet with +20 improved dry matter intake (DMI) by 11% and milk yield (MY) by 9% compared to a diet with -10 CAD. Blood bicarbonate (HCO_3^-)

increased linearly with increasing CAD indicating that higher CAD improved acid-base status. Because lactation diets typically contain more than +20 CAD, these results were considered more theoretical than practical. Studies with dietary CAD concentrations above +20 were needed. West et al. (1990) provided additional information when they evaluated diets with +2.5, +15, +27.5 and +40 CAD. They noted that increasing CAD from +2.5 to +27.5 improved DMI, milk yield and blood bicarbonate concentration. No additional improvement was observed in cows fed diets between +27.5 and +40 CAD. In another study by that group (West et al. 1991), diets with even higher CAD (+10, +21.7, +33.4 and +45.1) were fed. Increasing CAD increased DMI and blood pH linearly, and blood HCO_3 curvilinearly.

From these studies it appears that cation-anion mineral interrelationships have a significant influence on acid-base status and lactational performance of dairy cattle. But many questions still remain. Projects in this dissertation were designed to address those questions. Overall objectives were:

(1) to determine optimal dietary concentrations of Na, K and Cl and CAD over a wide range of concentrations;

(2) to identify and quantify the influence of Na, K and Cl interrelationships and CAD on acid-base status and lactational performance;

(3) to determine the influence of different commercial sources of Na, K and Cl; and,

(4) to characterize physiological responses to the dietary K x Cl interrelationship that was discovered during the project.

CHAPTER 2
LITERATURE REVIEW

Review of Na, K and Cl Metabolism

This review will focus on literature related to the hypothesis that cation-anion mineral interrelationships influence acid-base status and lactational performance of dairy cattle. The chemical classifications; function, absorption and excretion; requirements and recommendations of Na, K and Cl will be reviewed. This will be followed by a discussion of the influence of dietary Na, K and Cl on acid-base status and lactational performance of dairy cattle.

Chemical Classification

Nutritionists refer to Na, K and Cl as macrominerals because they are required in greater quantities in the diet and are present at higher concentrations in animal tissue than trace minerals (NRC, 1989). Chemically classified as alkali metals, Na and K occur mainly as ions in biological systems. They possess a single valency electron which is bound weakly. A feature of Cl, which is a halogen, is the propensity for its atoms to gain electrons, especially in the formation of the alkali salts NaCl and KCl.

Function, Absorption and Excretion

Sodium. Sodium in the mammalian body occurs primarily in the extracellular fluid and in bone (Aitken, 1976). The exchangeable fraction is responsible for regulating extracellular fluid volume and acid-base status (McKeown, 1986). In addition, Na is involved in nerve impulse transmission, muscle contraction and regulation of plasma and cellular water and ion content (Lunn and McGuirk, 1990). Sodium also plays a critical role in the elaborate Na-K adenosine-triphosphatase enzyme (ATPase) responsible for creating the electrochemical gradient required to orchestrate cellular transport.

The idea of a Na pump in the cell membrane was introduced by Dean (1941) in his classical paper on the theories of electrolyte equilibrium in muscle. Skou (1957) described an ATPase that was Na and K dependent. He suggested that the enzyme might be an essential part of a Na-K pump responsible for maintaining high K and low Na in cells. It was not until 1979 when it was proven "beyond reasonable doubt" that the pump was actually a Na-K pump, coupling movement of three Na^+ to the outside of the cell for two K^+ to the inside of the cell (Neilsen, 1979). Considerable information has since been accumulated, demonstrating that the Na-K ATPase is a transmembrane substrate of the Na-K pump.

The Na-K pump is universal to cellular physiology and enables all eukaryotic cells to function. The potential energy of the ionic chemical gradient created by the Na-K pump drives cellular transport. Other nutrients are transported by gradients set up by the Na-K pump. Phosphate, amino acids, and glucose are moved into cells and H^+, Ca^{++},

HCO_3^-, K^+ and Cl^- are moved out of cells by these gradients (Lechene, 1988).

Sodium salts are very soluble within the gastrointestinal tract (Peeler, 1972). Most dietary sources of Na are presumed to be nearly completely available. O'Dell and Savage (1966) indicated that acetate, citrate and carbonate forms of Na were more effective than NaCl in stimulating growth of chicks. Cation-anion interrelationships and not biological availability of Na salts may have been responsible for these effects (to be discussed in a later section). Because Na also exists in non-exchangeable form within the crystalline fraction of bone (Edelman et al., 1954), the Na in animal by-product feedstuffs may not be as bioavailable as in other feed sources of Na. The ARC (1980) estimated that 91% of consumed Na was absorbed.

A close association between Na, K and Cl excretion exists. The kidney is the primary organ regulating excretion of these ions (Lunn and McGuirk, 1990). Sodium is the primary effector of ion excretion and changes in reabsorption are the primary determinants of Na excretion. Hormone systems, including aldosterone, renin-angiotensin and arterial natriuretic factor, work with receptors in various tissues to monitor Na concentration, which in turn controls fluid volume, blood pressure and renal processing of the other ions.

The specific sequences of events related to hormonal regulation are as follows. Upon detection of reduced pressure in the renal afferent-arterioles, reduced plasma volume and/or reduced plasma Na, the juxtaglomerular cells of the kidney secrete rennin. Rennin is an enzyme that cleaves a decapeptide, angiotensin I, from a plasma alpha-globulin,

angiotensinogen. A converting enzyme present in plasma then cleaves a dipeptide from angiotensin I and forms angiotensin II. Angiotensin II, which also is stimulated by adrenal corticotrophic hormone and excessive plasma K, mediates the release of aldosterone by arcuate cells of the zona glomerulus in the adrenal cortex. Aldosterone acts in the cortical collecting tubule to increase luminal permeability to Na and K and to increase the peritubular Na-K/ATPase activity (Kleinman and Lorenz, 1989; Lunn and McGuirk, 1990). The net effect is resorption and retention of Na and excretion of K.

Potassium. In contrast to Na, most of the K in the mammalian body is inside cells. Potassium is the chief intracellular cation and most concentrated mineral element in milk (Hemken, 1983). Potassium functions in acid-base and osmotic pressure regulation, O_2 and CO_2 transport, and nerve and muscle contractions and is essential to many enzyme reactions.

There is considerable variation in red blood cell (RBC) K reported in the literature. Values between 22 and 106 meq/kg have been reported for bovine RBC K (Aitken, 1976; Hemken, 1983). The wide variation may be due to the method of measuring RBC K. Potassium in the RBC has been measured either directly by separating red cells from plasma in a centrifuged sample of whole blood, or indirectly by estimating K in whole blood and in plasma, measuring packed cell volume, and then calculating K by taking the difference. The first method is usually more accurate because indirect measures can lead to large errors. However, centrifugation may not give complete separation of plasma from RBC so it is necessary to wash the cells with an isotonic solution that

removes excess plasma but maintains viability of the RBC. If blood is handled such that cells are not ruptured, washing may be the preferred method to estimate RBC K (Aitken, 1976). There also are different ways to express RBC K. The concentration of K in the RBC can either be expressed as meq/kg dry cells or meq/L wet cells.

Potassium in feed occurs as simple ions, which upon consumption, are readily solubilized within the gastrointestinal tract and are almost completely absorbed. It was concluded by Hemken (1983) that the true digestibility of K is relatively high (95% or higher) for most feedstuffs. Dietary K from both inorganic and organic sources is utilized efficiently (Peeler, 1972).

Urine is the main excretory route for dietary K. As discussed previously this is primarily controlled by aldosterone. Blood pH also affects urinary K excretion (McGuirk and Butler, 1980). At the onset of an alkalotic condition, intracellular hydrogen protons are exchanged with plasma K as part of the regulatory mechanisms that control blood pH. In the renal tubules, a large gradient exists between intracellular and luminal fluid (urine) K. This gradient causes K to leave the tubule cells and enter the urine. The importance of K administration in the treatment of metabolic alkalosis in ruminants has been demonstrated (McGuirk and Butler, 1980). The feces, which are another route of eliminating K, can contain both undigested and endogenous K. Paquay et al. (1969b) estimated that 2.2 g of K per kg DMI were eliminated through the feces.

Chloride. Chloride comprises the major anionic component of mammalian extracellular fluid. Throughout the body, the metabolism of

Cl is associated intimately with Na and K function. Chloride functions in the maintenance of acid-base equilibrium, transport of O_2 and CO_2, and formation of gastric HCl (NRC, 1989). Most texts have described the role of Cl in maintaining ionic and fluid balance as passive to that of Na and K. However, research with lactating dairy cattle has shown that during Cl deficiency, the ion functions independently to mediate Cl conserving mechanisms (Fettman et al., 1984b). Cows fed a deficient amount of Cl in the study of Fettman et al. (1984b) and in several other studies conducted at Cornell University were able to conserve Cl by reducing excretion in urine, feces and milk. It also was demonstrated that cows may have a specific appetite for Cl. Cows fed diets with a deficient amount of Cl sought out and consumed more salt block than cows fed diets with adequate amounts of Cl.

As a strong ion, Cl is always dissociated in solution (Stewart, 1981). Although not extensively studied, true digestibility of Cl from all feed sources is presumed to be very high (i.e. 90% or greater). The ARC (1980) used 91% as the absorption efficiency for Cl. Paquay et al. (1969a) found that Cl digestibility was not influenced by Cl intake but was negatively correlated with DMI, energy, and pentosan intake; and positively correlated with nitrogen and K intake. Although Cl digestibility is high, the concentration in feedstuffs varies considerably (Adams, 1975; Coppock and Fettman, 1977).

Absorption of Cl represents an interesting phenomenon. Thirty years ago, Sperber and Hyden (1952) showed that Cl was transported through the rumen against a large concentration gradient (normal ruminal fluid concentration may range from 10 to 30 meq/L, whereas plasma Cl

normally ranges from 90 to 110 meq/L). Therefore, Cl was assumed to be actively transported. Martens and Blume (1987) verified that Cl was actively co-transported with Na across the ruminal wall in sheep. The mechanism of co-transport could not be deduced. Chloride absorption in the upper small intestine is via passive diffusion; Cl follows Na along an electrical gradient (Coppock, 1986). In the distal ileum and large intestine, Cl absorption is exchanged with bicarbonate secretion. Bicarbonate ions may serve to neutralize acids produced by intestinal fermentation (Coppock, 1986).

Studies with steers and wethers have shown that approximately 98% of ingested Cl was excreted in the urine (Nelson et al., 1955). However, in several studies with lactating cows, a major portion of Cl consumed was excreted in the feces (Coppock, 1986). In general, negative ion concentration in extracellular fluid is regulated secondarily to regulation of positive ion concentration, and when the load exceeds maximum capability for reabsorption from the kidney, the excess is excreted in urine (Hilwig, 1976). A reciprocal relationship exists between Cl^- and HCO_3^- ions in the kidneys (Kleinman and Lorenz, 1989). Excretion of one Cl ion is coupled to reabsorption of one HCO_3^- (or vice versa, depending on systemic pH; Fischer et al., 1983).

Dietary Requirements and Recommendations

Methods of Determination

Requirements and recommended allowances of dietary Na, K and Cl have been established (NRC, 1989; ARC, 1980). Both factorial and empirical methods have been used in their determination.

Dietary requirements have been established by the factorial method. This method involves the estimation of the amount (i.e. grams) of an element that is required for body growth, pregnancy and lactation. These factors are then added to the maintenance requirements needed to offset incomplete absorption, endogenous secretion into the gastrointestinal tract, urinary excretion, and insensible losses through dribbled saliva, sweat, tear ducts and skin. The factorial method thus establishes net requirements (ARC, 1980).

Dietary recommendations have been defined as the diet concentration (i.e., percentage or parts per million) of a mineral needed to achieve some predictable animal response (Beede, 1991). These have been established using the empirical method, which consists of adding graded quantities of a mineral to the diet and then measuring the selected response.

Beede (1991) suggested that mineral requirements determined by the factorial method are conceptually sound but have limited practical value. This is because requirements may be determined with animals in varied physiological states, fed with varied numbers of feeds. Dietary recommendations include a margin of safety that accounts for variation due to the physiological state of the animal and due to different feed sources and combinations of feeds. The NRC (1989) provides tables for both requirements and recommendations for minerals. The following will provide a summary of recent dietary recommendations for Na, K and Cl.

Recommended Dietary Concentrations of Na, K and Cl

Sodium. The NRC (1989) recently updated mineral requirements and made considerable changes in recommendations for dietary Na, K and Cl. Due to the historical use of common salt (NaCl) to meet Na and Cl needs, recommendations were reported previously as a general recommendation for common salt (NaCl). However, an important separation was made (NRC, 1989) between the requirements for "salt" and the individual components of salt (Na and Cl). This refinement should prove useful because physiological needs are related to the individual ions, not the salt molecule (which of course dissociates into Na and Cl upon consumption). Also, because of the trend to feed dairy cows $NaHCO_3$ as a source of Na (instead of NaCl), this separation should help eliminate potential underfeeding of Cl.

The NRC (1989) lists .18% as the recommended Na concentration for lactating dairy cattle. However, this concentration may not maintain Na balance. The NRC (1989) states that data from balance experiments involving lactating cattle fed many different diets showed that Na balance was negative when dietary Na was below .20% DM. Lactational performance may be enhanced with Na concentrations above .18%. Florida researchers found that lactating dairy cattle consumed more feed and produced more milk when fed diets with higher than recommended concentrations of Na. In a cool weather study, Mallonee et al. (1982) fed three concentrations of Na: .16, .42, and .70% of diet DM. Several cows fed .16% Na exhibited classical signs of Na deficiency within 2 weeks. Cows fed diets .42% Na consumed the most feed, whereas cows fed diets with .7% Na produced the greatest quantities of milk (Na results

were averaged across K concentrations, another variable in the study). Because diets were not isochloridic in that study, responses may not have been wholly due to Na.

In a warm weather study with equal concentrations of dietary Cl (Schneider et al., 1986), increasing dietary Na above NRC (1989) recommendations (from .18 to .55% using either NaCl or $NaHCO_3$ as the source of Na) improved MY and FCM yield. Other researchers have reported increased MY and milk composition with increasing dietary Na, but have attributed those improvements to ruminal buffering by HCO_3^- rather than to Na (Erdman et al., 1980; Rogers et al., 1982a and 1982b; Kilmer et al., 1981). Schneider et al. (1986) suggested that lactational responses to increasing dietary Na may be due in part to Na per se and not wholly to HCO_3^-. Subsequent calculations demonstrated that 60% of the FCM yield response was due to Na. Additional research will be needed to better define the correct amount of Na to feed to lactating dairy cattle.

Potassium. The NRC committee increased the recommendation for dietary K in their most recent update (NRC, 1989). Minimum recommendations were increased from .8 to .9% for average milk production and to 1.0% for high production. Factors responsible for the increase included: (1) inclusion of higher milk production categories; (2) potential use of by-product feeds with lower concentrations and reduced bioavailabilty of K; and (3) increased need during heat stress. Historically, dietary K recommendations have been the subject of debate.

Recommended concentrations of dietary K (DM basis) for lactating dairy cattle have included .5% (Ward, 1966), .7% (NRC, 1971), .8%

(Dennis et al., 1976; Dennis and Hemken, 1978; NRC, 1978; Erdman et al., 1980), .9% (NRC, 1989), 1.0% (Linsner, 1980) and 1.2% (Bolenbaugh, 1977). Even greater concentrations have been suggested for cows strained by heat and humidity (Mallonee et al., 1985; Schneider et al., 1984b, 1986; West et al., 1987b). It is possible that some of the discrepancies between published K recommendations were due to differing dietary Cl concentrations used in the various studies. Paquay et al. (1969b) found a close correlation between K and Cl in the urine and suggested that adequate Cl was needed to accompany urinary excretion of excess dietary K. Dietary Cl concentrations in most studies that investigated K were not reported making it difficult to evaluate K x Cl interactions. In retrospect, analyzing diets for Cl would have been valuable.

Beede et al. (1983) reviewed the relationships of K nutrition and heat stress in lactating cows. During heat stress, dietary K needs are increased. Losses of K via sweat and dribbled saliva increase and are compounded by reduced feed and K intake. Cows without the benefit of shade responded to increasing dietary K (from .66 to 1.08%) more than did cows under shade. Increasing dietary K (from .93 to 1.29 and 1.53% K) improved lactational performance during hot weather in Texas (West et al., 1987b).

As mentioned previously, alkalosis can increase urinary excretion of K. During heat stress, cows pant to cool themselves because panting alters alveolar ventilation and CO_2 is eliminated faster than it is produced. In addition, partial pressure of CO_2 (pCO_2) is lowered and blood pH rises (Collier et al., 1982). Two factors associated with this

heat-stress-induced-alkalosis contribute to a need for increasing dietary K. First, decreased pCO_2 reduces renal tubular acid secretion and can cause compensatory loss of alkali reserve (i.e. to maintain electroneutrality of the urine, K^+ replaces H^+). Second, at the onset of alkalosis, intracellular H^+ ions are exchanged with plasma K as part of the regulatory mechanisms that control H^+. In the renal tubules, a large gradient exists between intracellular and urinary K. This gradient causes K to exit the tubule cells and become excreted in urine.

Chloride. The Cl recommendation recently was set at .25% of diet DM (NRC, 1989). This guideline is based on work designed to establish the minimal requirement of Cl and role of Cl in acid-base status (Fettman et al., 1984b; Coppock, 1986). Fettman et al. (1984b) concluded that the requirement for Cl was above .1%. In their published figures, average daily milk production for cows fed the .45% Cl diet appeared lower but was not different from cows fed the .27% Cl diet. Low dietary Cl concentrations (.1% Cl) led to subclinical primary-hypochloremic secondary-hypokalemic metabolic acidosis as well as reduced feed and water intake, body weight, and milk production.

Coppock (1986) suggested that about .2% Cl is required for a lactating cow in midlactation. He suggested that .25% would be too low during peak lactation and negative energy balance. Underwood (1981) proposed that the Cl requirement should be substantially higher than the Na requirement because cow milk contains more than twice as much Cl as Na. Other factors that may affect Cl needs include type of diet, rate of growth, pregnancy and heat stress (Coppock, 1986).

Chloride values of feeds are not always reported in feed tables, appear highly variable, and are missing from many ration balancing programs. Commercial feed analysis laboratories often only report feed Cl concentrations upon special request (Coppock, 1986; Chandler, 1988).

Influence of Cation-Anion Interrelationships on Acid-Base Status

General Principles of Acid-Base Status

To understand how macromineral cation-anion interrelationships influence acid-base status, a review of acid-base status is essential. Concentration of H^+ ($[H^+]$) in extracellular fluid (ECF) is found in very small quantities--one tenth to one hundred millionth of an eq/L (approximately 40 neq/L, i.e., pH 7.4). This is a trillion times less concentrated than water. Although $[H^+]$ is minuscule, $[H^+]$ is critical to living systems for several key reasons (Stewart, 1981; Morris, 1986):

(1) because H^+ atoms are so small they have a large charge density and a large electric field gradient,

(2) hydrogen bonds are important in determining molecular structure and configuration,

(3) enzyme activity is very sensitive to $[H^+]$,

(4) $[H^+]$ turnover amounts to more than 150 mole/d in the human, which is more than any other metabolite, and,

(5) because H_2O can dissociate into H^+ there is an infinite supply of H^+ ions.

Regulation of Acid-Base Status

The regulation of pH in body fluids ranks high among the homeostatic priorities. Boron (1986) probably said it simplest when he stated that "virtually every biological process is pH sensitive." Stability of the $[H^+]$ is maintained by a number of biological buffering mechanisms involved in various systems of the body (Hilwig, 1976).

A series of chemical buffer systems (bicarbonate-carbonic acid buffer pair, phosphates, plasma proteins, and hemoglobin) respond to short-term perturbations. These systems act to neutralize acids or bases produced by tissues or derived exogenously. Buffering mechanisms in the respiratory system act to provide the main route of elimination of CO_2 and are of prime importance in maintaining the HCO_3:pCO_2 ratio, a determinant of extracellular pH. The third method of $[H^+]$ regulation is through renal elimination of non-volatile acids and bases (Hilwig, 1976).

Electrolytes and Strong Ions

Many substances, when dissolved in solution, dissociate into ions. These substances are chemically defined as electrolytes because they can conduct electricity. Strong electrolytes are by definition (Stewart, 1981) completely dissociated, whereas weak electrolytes change their degree of dissociation. Most substances that contain Na, K and Cl are strong electrolytes and dissociate completely and rapidly in solution. Other macromineral sources have variable dissociation constants (Stewart, 1981). Because they arise from strong electrolytes, Na, K and

Cl are classified as strong ions and are ionized in solution. A physical law that applies to ionic solutions is the law of electrical neutrality (Stewart, 1981). Biological fluids must be maintained in an electrically neutral environment. The sum of positive charges must equal the sum of negative charges. Dietary electrolytes contribute positive and negative charges to the body and therefore can alter electrical balance, acid-base status, and subsequent animal performance. Because ions react according to charge (valence) they should be expressed in moles (or equivalents) of charge rather than moles of atoms. This is easily demonstrated by the reaction of one mole (1 g) of H ions with one mole (35 g) of Cl ions. The reactants have the same number of atoms but are drastically different in weight. When considering the influences that strong ions and cation-anion interrelationships have, concentrations should be converted to milliequivalents (meq; measure of ionic charge).

The Strong Ion Difference

Peter Stewart, the late muscle physiologist, quantified $[H^+]$ in body fluids and demonstrated mathematically that $[H^+]$ of the blood depends only on three variables: the total weak acid; pCO_2 in the blood; and the difference between strong cations and strong anions in blood (strong ion difference: SID). The concept of SID is becoming widely used in veterinary medicine (Eicker, 1990) and is notably similar to the dietary cation-anion difference concept used by animal nutritionists (to be discussed later).

Specific Effects of Macromineral Salts on Acid-Base Status

Nutrient metabolism results in the degradation of nutrient precursors into strong acids and bases. During normal metabolism the flux of H^+ is great. In typical rations fed to dairy cattle, inorganic cations exceed dietary inorganic anions by several meq per day. Carried with excess dietary inorganic cations are organic anions which can be combusted to HCO_3^-. Therefore, a diet with excess inorganic cations relative to inorganic anions is alkaline (Austic, 1988). However, for a dietary mineral salt to affect acid-base status it must satisfy the following three criteria:

(1) the mineral salt must be dissociated and solubilized in the gastrointestinal tract.;

(2) upon dissociation and solubilization it must be absorbed; and,

(3) after absorption, any non-mineral portion must be metabolized such that $[H^+]$ in blood increases or decreases.

Although NaCl meets the first two criteria, it does not affect acid-base status because neither Na nor Cl are metabolized. Thus NaCl has a neutral effect on acid-base status (disregarding indirect solute effects). Examples of mineral salts that meet these criteria include ammonium chloride, an acidogenic agent, and sodium bicarbonate, an alkalogenic agent. Examining their chemical reactions upon absorption illustrates how each affect acid-base status.

Ammonium chloride (NH_4Cl). When NH_4Cl reaches the gastrointestinal tract it first dissociates into NH_4^+ and Cl^- ions. The NH_4^+ ions are further metabolized into two NH_3 molecules and one H^+ ion.

Two NH_3 molecules combine with CO_2 (in the urea cycle) and form urea. The steps in the reaction are:

(1) $2\ NH_4Cl \quad <------> \quad 2\ Cl^- + 2\ NH_4^+$

(2) $2\ NH_4^+ \quad ------> \quad 2\ \underline{H^+} + 2\ NH_3$

(3) $2\ NH_3 + CO_2 \quad ------> \quad \text{urea} + H_2O$

Because H^+ ions in blood are increased (underlined in the above reactions), NH_4Cl is an acidogenic salt. The Cl^- ion is fixed but NH_4^+ is metabolized with the net addition of two H^+ ions. The metabolism of the NH_4^+ portion of NH_4Cl is the key reason why NH_4Cl is considered an acidogenic salt. Note that some texts refer to Cl as an acidogenic ion. Chloride is only acidogenic when it is associated with a metabolizable cation (or with H as in HCl). Note that $(NH_4)_2SO_4$ affects on acid-base status are similar to that of NH_4Cl.

Sodium bicarbonate ($NaHCO_3$). Another example of a salt that affects acid-base status is $NaHCO_3$, an alkalogenic salt, commonly fed to ruminants to buffer ruminal pH. Upon metabolism of $NaHCO_3$, the Na^+ ion is fixed and will not affect acid-base status. But the HCO_3^- is metabolized to H_2O and CO_2 with the net removal of one H^+ ion.

(1) $NaHCO_3 + H_2O <-----> Na^+ + \underline{OH^-} + HCO_3^-$

(2) $HCO_3^- + H^+ <-----> H_2O + CO_2$ (expired in lungs).

Ammonium chloride is acidogenic and $NaHCO_3$ is alkalogenic because the non-mineral portion of these salts are metabolized. It is not the minerals per se that are acidogenic or alkalogenic but rather the non-mineral portions that contribute or consume H^+.

Calcium chloride ($CaCl_2$). One additional mechanism exists to explain how a mineral salt affects acid-base status. In this case only a portion of the mineral is absorbed. An example of a type of salt that functions in this manner is $CaCl_2$. Upon consumption of $CaCl_2$, only a small percentage of Ca is absorbed (Peeler, 1972). To maintain neutrality in the digestive tract, unabsorbed Ca^{++} ions combine with HCO_3^- to form the precipitate $CaCO_3$, which is then excreted in the feces.

$$CaCl_2 + HCO_3^- \text{<-----> } 2\ Cl^- + \underline{H^+} + CaCO_3 \text{ (precipitate).}$$

Calcium chloride is an acidogenic salt because it increases systemic H^+. However, the reason $CaCl_2$ is acidogenic is due to incomplete absorption of Ca. Other acidogenic salts which react and behave similarly are $MgCl_2$, and Mg_2SO_4.

Note that the pH of a salt (or feed) should not be used to predict the effect it will have on acid-base status. For example, a chemically neutral salt such as $CaCl_2$ results in acidosis, whereas Na_2HPO_4, chemically an acid, results in alkalosis.

Nutritional Concepts Related to Cation-Anion Interrelationships

Leach (1979) and Mongin (1980) reviewed nutritional concepts related to cation-anion interrelationships. Historically, nutritionists intuitively knew it was difficult to evaluate the effect of one macromineral without considering the influences of others. Early concepts evaluated total ash, mineral ratios, and differences among two or more of the macrominerals.

Acid or Alkaline Ash

Nutritionists first investigated the alkalinity and acidity of the diet under the acid- or alkaline-ash concept (Shohl, 1939). It was recognized that human food either had an acid or alkaline ash. When food is metabolized in the body, organic anions, such as acetate, citrate, malate, etc., are oxidized. Inorganic cations originally associated with these organic anions remain. Because organic anions can buffer H^+ ions generated through metabolism (see next section), a food with a large amount of organic anions (and thus inorganic cations) was considered alkaline. The pH of the ash represented the acid or alkaline nature of human food.

Ratios

Later concepts used to evaluate the effects of macromineral interrelationships involved ratios of two or more minerals. Leach (1979) listed several macromineral ratios that were used to express the influence of macromineral interrelationships on acid-base status and animal performance. Apparently, the use of ratios were not widespread. A professor from the University of Florida Poultry Science Department advises his students that mineral ratios can be misleading (R.D. Miles, personal communication). As an example he cites the Na/Cl ratio mentioned by Leach (1979). A typical lactating dairy cattle diet might have about 15 meq of Na and 7.5 meq of Cl (2:1 ratio). The difference between meq from Na and Cl is 7.5 meq (15 meq from Na minus 7.5 meq from Cl). If the meq from each ion were doubled in the diet, the ratio would still be 2:1 but the difference would grow to 15 meq. The additional

7.5 meq Na could impact acid-base status, yet, this would not be detected by calculating the ratio. Other researchers recognized the shortcoming with ratios and began to use differences.

Dietary Undetermined Anion

One of the most common difference expressions used was the "dietary undetermined anion" (DUA) (Austic, 1988). With DUA, cations and anions are classified as separate entities. All seven macrominerals are considered in the expression:

DUA = meq [(Na + K + Ca + Mg) - (Cl + P + S)]/100 g diet DM.

Under the law of electrical neutrality (Stewart, 1981), the positive and negative charges in the diet need to balanced electrically. If the mineral cations in the DUA expression exceed the mineral anions, then some "undetermined" anions must be present in the diet because by definition and in actuality the feed is electrically neutral. These undetermined anions presumably consist of carboxylate groups of organic compounds, such as bicarbonate, citrate, lactate, and fatty acids (Austic, 1988). Because dietary organic anions are precursors to HCO_3^-, they contribute alkali. Consequently, DUA measures alkaline potential of the diet. Because trace mineral cations and anions do not represent large concentrations in the diet, ionic contributions from trace minerals are ignored in the DUA expression. A problem with including the multivalent macrominerals (Ca, Mg, P, and S) in the DUA expression for ruminants, relates to the variable and incomplete bioavailability of these ions compared to Na, K and Cl (Peeler, 1972).

Cation-Anion Difference (CAD)

The expression most widely used in ruminant nutrition is the monovalent cation-anion difference (CAD) expressed as Na + K - Cl (in meq/100 g diet DM). This expression is considered superior because it comes closest to representing feed ions that are completely dissociated, solubilized from their respective salts, and absorbed into the body.

To calculate CAD, mineral concentrations are first converted to milliequivalents using the following equation:

$$\text{meq/100 g} = \frac{(\text{milligrams})(\text{valence})}{(\text{atomic weight})}$$

As an example, the CAD value of a diet with .18% Na, .9% K and .25% Cl (minimum recommendation for lactating cattle; NRC, 1989) will be calculated. There are 180 mg Na (.18% = .18 g/100 g or 180 mg/100 g), 900 mg K and 250 mg Cl per 100 g diet DM. Therefore this diet contains:

$$\text{meq Na} = \frac{(180\text{ mg})(1\text{ valence})}{(23\text{ g atomic weight})} = 7.8\text{ meq Na}$$

$$\text{meq K} = \frac{(900\text{ mg})(1\text{ valence})}{(39\text{ g atomic weight})} = 23.1\text{ meq K}$$

$$\text{meq Cl} = \frac{(250\text{ mg})(1\text{ valence})}{(35.5\text{ g atomic weight})}. = 7.0\text{ meq Cl}$$

The next step is to sum the meq from the cations and subtract the meq from the anions:

meq (Na + K - Cl) = 7.8 + 23.1 - 7.0 = + 23.9 meq/100 g diet DM.

Therefore, a diet for a lactating dairy cow containing the minimum recommended concentrations of Na, K and Cl has a CAD of +23.9.

This method to calculate CAD is straight forward due to the single charge present on the monovalent ions. The incomplete dissociation of other macrominerals affects charge valency and thus acid-base status (Dweyer et al., 1985). For example, P, a divalent anion, actually is assigned a valence of 1.8 (instead of 2) because in blood, P exists as both $H_2PO_4^-$ and $HPO_4^=$. Because approximately 80% exists as the divalent anion, the valance is assumed to be 1.8 [(.8 x 2) + (.2 x 1) = 1.8]. An assumption to the degree of dissociation (which is affected by several factors) is critical to the estimated influence of P on acid-base status.

Cation-Anion Interrelationships and Effects of Cation-Anion Difference on Animal Performance

Sodium, Potassium and Chloride Interrelationships

Because Na, K and Cl function together to maintain acid-base equilibrium, it is likely that they have interrelated effects on animal performance. Studies have been conducted on requirements for each mineral, but data on their interrelationships are limited.

Studies from other species suggest that optimal concentrations of each of these macrominerals depends upon relative concentrations of the others (Fontenot et al., 1960; Scott, 1970; Johnson and Karunajeewa 1985). Studies conducted with lactating dairy cattle on the Na x K interaction have had variable results. Some have found that the response to dietary Na concentration depended on the associated concentration of dietary K (Schneider et al., 1986), whereas others have found that response to dietary Na did not depend on dietary K (Erdman et

al., 1980; O'Connor et al., 1988). Results may have been related to whether or not Cl concentrations were equalized among treatments.

Most published dietary Na x K interaction effects were related to nutrient absorption which usually indicated a sparing of the two cations for each other. In poultry and rats, additional dietary Na spared a portion of the K requirement (Kumpost and Sullivan, 1966; Burns et al., 1953; Grunert et al., 1950). Fontenot et al. (1960) reported that additional dietary Na depressed K absorption in lambs. Increasing dietary K intake in sheep resulted in an increase in fecal Na (Suttle and Field, 1967). Scott (1970) found that high dietary K impaired intestinal absorption of Na and low dietary K increased urinary Na excretion in cattle. Campbell and Roberts (1965) reported that apparent intestinal absorption of Na in heifers was impaired by high dietary concentration of K but lower concentrations of K increased urinary loss of Na. Scott (1967) observed that an increase in the ruminal fluid concentration of one of these ions is accompanied by a reciprocal decrease in the other, resulting in an almost constant meq sum of Na plus K. Jackson et al. (1971) observed an Na x K interaction on microbial populations in the rumen.

In lactating cattle, previous studies on the interrelationships between dietary Na and K have not revealed a sparing effect of dietary Na and K for each other. Erdman et al. (1980) found no benefit of additional dietary Na (.52 vs. .31%) with either low (.42%) or adequate (.84%) dietary K. O'Connor et al. (1988) also reported no benefit on lactational performance due to additional dietary Na (.24 vs. .62%) with either 1.14 or 1.59% dietary K. Chloride was not equalized across diets

in those studies which could explain the lack of response. Martens and Blume (1987) observed that Na and Cl absorption in sheep was coupled by a dual co-transport mechanism for Na^+/H^+ and Cl^-/HCO_3^- which was related to ruminal K concentrations. An alteration in the relative amounts of dietary Na and K thus could affect acid-base status which in turn could affect lactational performance.

Coppock et al. (1982a) studied Na x Cl interactions with cows fed diets with Na:Cl ratios between 1:1 and 4:1 and did not find differences in MY or milk composition. Belgium researchers (Paquay et al., 1969) found a positive correlation between K and Cl in the urine of cows and suggested that the need for dietary K may be related to dietary Cl.

Cation-Anion Difference

The cation-anion concepts of Shohl (1939) appear to be creeping back into animal nutrition with the recent findings that dietary mineral interrelationships affect acid-base homeostasis. Mongin (1980) concluded that in order to maintain acid-base homeostasis, an animal needs to regulate input and output of acidity. Net acid intake can be extrapolated from the difference between dietary fixed cations and anions (i.e. those ions that are not metabolized during digestive or metabolic processes). Tucker et al. (1988a) discovered that acid-base balance and lactational performance were related to dietary CAD.

Nonruminants. The bulk of research on the CAD concept has been conducted with nonruminants. Effects of CAD on acid-base status and various health and growth responses in poultry have been investigated extensively. Diets with excessive mineral Cl and other anions, were

deleterious to acid-base status, growth rate, eggshell mineralization, and incidence of tibial dyschondroplasia (Austic, 1988). Mongin (1980) cited one study that tested various combinations of CAD on growth of broiler chicks. That data described a uniform bell shaped curve response to increasing CAD. Optimum performance was established at +25 to +30 CAD. Several diets were marginally deficient in Na, K or Cl which could explain some of the dramatic effects observed.

Growth responses to CAD also have been observed with swine. Yen et al. (1981) examined the effect of $CaCl_2$ and $NaHCO_3$ on feed intake and weight gain. They observed negative effects from feeding a diet with 4% $CaCl_2$ consistent with disturbances in acid-base status. When 2.03% $NaHCO_3$ was added to this diet, deleterious effects were eliminated. Patience et al. (1987) reported no difference in growth rate between 0 to +34 CAD but observed a depression with -8.5 CAD. Golz and Crenshaw (1990), reported maximal growth in growing pigs fed a diet with +23.8 CAD but an interaction between K and Cl influenced gains to a greater extent than CAD.

Dairy cattle. Most of the research with CAD in dairy cattle nutrition has been derived through studying milk fever problems and dry cow nutrition. It appears that significant progress can be made in combating hypocalcemia by considering CAD in the dry cow diet (Dishington, 1975; Lomba et al., 1978; Block, 1984; Oetzel, 1988; Wang and Beede, 1992). In general, studies have shown that when dry cow diets had negative CAD and when cows were in Ca balance, milk fever was diminished. In one study, milk fever was prevented 92% of the time when low CAD and high Ca was fed prepartum (Dishington, 1975). An increased

mobilization of bone Ca and enhanced absorption of Ca prepartum, which makes greater amounts of calcium available postpartum, appeared to be the cause.

Very few studies have examined the influence of CAD on lactating dairy cattle. Wheeler (1981) summarized several studies designed to investigate dietary buffers and could find no clear relationship between dietary CAD and animal performance. However, these studies were not designed specifically to evaluate CAD. Beef cattle did gain slightly less when CAD was below +10 than when CAD was above +77.

Coppock (1986) also reviewed the influence of dietary CAD on lactational performance of dairy cattle. In general it was noted that ruminants could withstand higher CAD than poultry or swine. In summarizing trials after the fact, he found that CAD had no influence between +10 to +40. Escobosa et al. (1984) studied effects of feeding either .23% NaCl, .23% NaCl plus 2.28% $CaCl_2$, or .23% NaCl plus 1.7% $NaHCO_3$. Diets had -14, +20 or +35 CAD. It was found that the excess Cl (-14 CAD) depressed feed intake and resulted in acidosis.

Tucker et al. (1988a) in Kentucky appear to have been the first to conduct a study specifically designed to evaluate the effect of CAD on acid-base status and lactational performance of dairy cattle. They compared diets formulated with -10, 0, +10 or +20 CAD. Twelve midlactation cows were assigned to three 4 x 4 Latin squares. Squares were arranged in a split-plot. Sub-plots represented either Na, K or Cl formulated diets and main-plots were the different CAD concentrations. A diet with +20 improved DMI 11% and MY 9% compared with a diet with -10 CAD. Blood HCO_3^- increased linearly with increasing CAD which indicated

an improvement in acid-base status with high CAD compared with low CAD. Because there were no effects due to square, they concluded that responses to increasing CAD were independent of specific Na, K or Cl effects. Because lactation diets typically contain greater CAD than +20, these results were primarily theoretical rather than practical. The next question that had to be answered was whether or not responses would continue to increase with diets above +20 CAD.

West et al. (1990) in Georgia answered part of this question when they evaluated diets with up to +40 meq/100 g diet DM. Their study used two 4 x 4 Latin squares blocked by environmental temperature (cool vs. hot). Separate squares included four Holstein and four Jersey cows. Diets contained +2.5, +15, +27.5 or +40 CAD. No effect of environment was reported but increasing CAD from +2.5 to +27.5 increased DMI, MY and blood HCO_3^-. These findings suggested that performance was depressed with lower CAD. At +27.5 CAD, negative effects were overcome. Above +27.5 CAD no additional improvement was attained.

In another study by this group (West et al. 1991), diets with even higher CAD (+10, +21.7, +33.4 and +45.1) were fed to a total of 16 lactating dairy cows during hot weather. Source of cation (Na or K) used to manipulate CAD also was compared. Increasing CAD increased DMI linearly, independent of Na or K source. Yield of 3.5% FCM was not affected by CAD or cation source. Milk fat concentration was greater with Na- compared with K-manipulated diets (3.92 vs. 3.62%). Blood pH increased linearly whereas blood HCO_3^- increased curvilinearly; there was no effect due to cation source on acid-base status. Their results indicated that increasing CAD improved DMI and acid-base status in a

manner consistent with other studies. In general, CAD was independent of a specific Na or K effect.

The influence of Na, K and Cl at constant CAD was evaluated by Tucker and Hogue (1990). Diets were formulated to provide +32 CAD in either: a basal diet (adequate in dietary Na, K and Cl), a basal diet containing an additional 1.17% NaCl, or a basal diet containing an additional 1.56% KCl. Fifteen midlactation cows were assigned to replicated 3 x 3 Latin squares. The KCl-fed cows consumed more DM and had lower milk fat percentage than NaCl-fed cows, but there were no differences in milk yield. It was concluded that dietary CAD was a more important determinant of dietary impact on systemic acid-base status than actual dietary concentrations of Na, K and Cl.

In summary, dietary CAD appears to exert its effect on lactational performance by altering acid-base status. When diets are below a certain CAD they likely contain insufficient inorganic cations in relation to inorganic anions. Amount of organic anions normally brought to the diet by inorganic cations are decreased and therefore cannot participate in buffering H^+. Acidosis occurs and normal milk production is compromised. From the limited data available, no specific dietary CAD recommendation can be made, but the optimum appears to be somewhere between +27.5 and +40. This may depend upon other factors such as the digestibility, fermentability and acid producing potential of the diet, dietary concentrations of other fixed ions, and rate of intake and production capacity of the animal.

The major focus of this research was to address Na, K and Cl nutrition of the lactating dairy cow. Determining the influence of

dietary Na, K and Cl interrelationships, particularly the three-way, dietary cation-anion difference interrelationship, on lactational and physiological responses was fundamental.

CHAPTER 3
INTERRELATIONSHIPS AMONG DIETARY SODIUM, POTASSIUM AND CHLORIDE: EFFECTS ON ACID-BASE STATUS, MINERAL METABOLISM AND LACTATIONAL PERFORMANCE OF DAIRY CATTLE

Introduction

Twenty years ago Jacobson et al. (1972) suggested that mineral interrelationships are the least understood and most promising area of dairy cattle mineral nutrition. The lactating dairy cows requirement for each mineral has been established, but information on mineral interrelationships is limited. Because Na, K and Cl function together to maintain fluid balance, osmotic regulation, and acid-base equilibrium (Kleinman and Lorenz, 1989), it is likely that they have interrelated effects on animal performance.

Studies from other species suggest that optimal dietary concentrations of these minerals depend on relative concentrations of the others (Fontenot et al., 1960; Scott, 1970; Johnson and Karunajeewa 1985). For lactating dairy cattle, there have been studies that have found Na x K interactions (Schneider et al., 1986) but there also are studies that have found no Na x K interaction (Erdman et al., 1980; O'Connor et al., 1988). Coppock et al. (1982a) studied Na x Cl interactions with cows fed Na:Cl ratios between 1:1 and 4:1 (percent of DM) but did not detect differences in milk yield or milk composition. Paquay et al. (1969b), however, found a positive correlation between K

and Cl in the urine of cows and suggested that the need for dietary K may be related to concentration of dietary Cl.

Mongin (1980) concluded that in order to maintain acid-base homeostasis, an animal needs to regulate input and output of acidity. Net acid intake can be extrapolated from the difference between dietary fixed cations and anions (i.e., those ions that are not metabolized during digestive or metabolic processes). Tucker et al. (1988a) showed that acid-base status and lactational performance were related to fixed cation-anion difference calculated as meq (Na + K - Cl)/100 g diet DM (CAD).

Objectives of the present study were three-fold: (1) to determine optimal dietary concentrations of Na, K and Cl, (2) to determine if optimal dietary concentrations of Na, K and Cl were interrelated, and (3) to determine if dietary CAD was related to lactational and physiological responses.

Materials and Methods

Management

Forty-eight midlactation Holstein cows were fed diets (Tables 3-1 and 3-2) twice daily at 0800 and 1400 h. An electronic feeding system (American Calan, Inc., NorthWood, NH) was used to measure daily feed intake and refusals of individual cows. Total mixed diets (Table 3-1) were made by combining corn silage with cottonseed hulls and concentrate immediately before each feeding which was delivered in a mobile mixing and feeding unit with electronic scales (American Calan, Inc.,

TABLE 3-1. Ingredient composition of basal diet.

Item	Percent of DM
Corn silage	40.00
Ground yellow corn	31.00
Corn distillers dried grains	15.00
Cottonseed hulls	5.50
Hydrolyzed feather meal	2.50
Urea	1.00
Vitamin-mineral premix[1]	.75
Treatment mineral mixture[2]	4.25

[1]Contained Ca 29%, P 13%, Mg 2%, S 1%, Mn .22%, Zn .33%, Cu .12%, I .007%, Se .003%, Co .0002%, Vitamin A 110,000 IU/kg, Vitamin D_3 99,000 IU/kg, and Vitamin E 330 IU/kg.

[2]Contained various combinations of $CaCO_3$, MgO, KCl, NaCl, $CaCl_2$, Na_2CO_3, K_2CO_3 and SiO_2 (washed sand) in proportions designed to obtain treatment formulations.

TABLE 3-2. Analyzed chemical composition of concentrate, corn silage and TMR (DM basis).[1]

	Concentrate		Corn Silage		TMR[2]
Nutrient	Mean	SEM	Mean	SEM	Mean
CP, %	21.2	.28	8.65	.22	16.18
ADF, %	14.8	.34	36.2	1.62	23.36
Ca, %	1.18	.035	.35	.02	.85
P, %	.50	.012	.21	.006	.38
Mg, %	.31	.008	.27	.01	.29
S, %	.26	.002	.15	.002	.22
Fe, ppm	379.0	25.0	161.0	52.0	292.0
Zn, ppm	74.5	1.76	39.8	1.17	60.6
Cu, ppm	16.1	.9	6.67	.3	12.3
Mn, ppm	49.1	1.45	31.6	3.8	42.1
NE_L, mcal/kg[3]	1.70	.002	1.43	.03	1.59

[1]Means and SEM for each component were calculated from analyses of composite samples taken throughout the experiment.

[2]TMR calculation based on 60:40 concentrate:corn silage (DM basis); cottonseed hulls were part of concentrate when sampled.

[3]Value calculated from chemical analysis.

NorthWood, NH). Cows were fed so that 5 to 10% of feed (as-fed basis) remained at 0630 h. Remaining feed was removed and weighed prior to morning feeding. All cows were fed the same corn silage-alfalfa hay based TMR during a 1 month preliminary period. Cows were housed in a freestall barn and were randomly assigned to one of four equal sections within the barn. Cows had access to exercise lots and drinking water at all times. Milking was at 0500 and 1600 h.

Treatments

Each cow received a different dietary treatment in each of four consecutive 28-d periods from early March to late June. Cows were assigned treatments according to a partially balanced incomplete block design. Treatments consisted of dietary combinations of Na, K and Cl, defined according to a three-factor second-order rotatable central-composite design (CCD; Figure 3-1; Table 3-3; Khuri and Cornell, 1987). This CCD consisted of eight treatments arranged in a 2 x 2 x 2 factorial (high and low concentrations of Na, K and Cl), plus six 2 x 3 axial treatments (very high and very low concentrations of Na, K and Cl), and one center point treatment (middle concentration of Na, K and Cl). With this CCD, only 15 treatments were required compared to the 27 that are required in a standard factorial arrangement of treatments. In addition to being more economical to run than a 3 x 3 x 3 factorial, this CCD allowed each factor to be studied at five instead of only three concentrations (Khuri and Cornell, 1987).

Treatment combinations of Na, K and Cl were selected by first defining the center point (treatment 15; .55% Na, 1.4% K and .8% Cl).

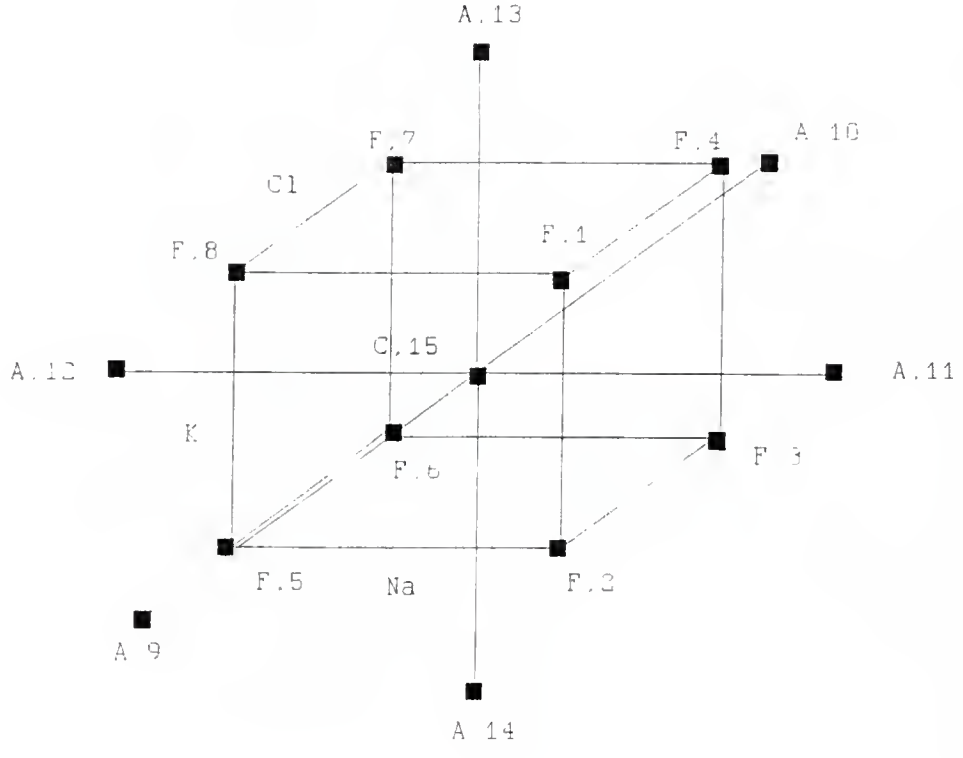

Figure 3-1. Three-factor central composite design used to evaluate interrelationships among dietary Na, K and Cl concentrations. Center point, factorial, and axial treatment design points are labeled, numbered and indicated by ■. C = center point (middle concentration of Na, K and Cl), F = 2 x 2 x 2 factorial points (low and high concentration of Na, K and Cl), and A = axial points (very high and very low concentration of Na, K and Cl). See Table 3-3 for dietary concentrations.

TABLE 3-3. Dietary concentrations of Na, K, Cl and calculated cation-anion difference (CAD) of experimental diets (% of diet DM).

	Dietary concentration (DM basis)[1]			
Treatment	Na,%	K,%	Cl,%	CAD[2]
1	.75	1.7	.5	62.0
2	.75	1.1	.5	46.7
3	.75	1.1	1.1	29.8
4	.75	1.7	1.1	45.1
5	.35	1.1	.5	29.3
6	.35	1.1	1.1	12.3
7	.35	1.7	1.1	27.7
8	.35	1.7	.5	44.6
9	.55	1.4	.3	51.3
10	.55	1.4	1.3[3]	23.1
11	.89	1.4	.8	52.0
12	.21[3]	1.4	.8	22.4
13	.55	1.9	.8	50.0
14	.55	.9	.8	24.4
15	.55	1.4	.8	37.2

[1] Unless otherwise indicated, concentrations shown were calculated values required to provide Na, K, and Cl concentrations for a second-order three-factor central composite design. Actual analyses were .31, .34, .57, .74, .85; .86, 1.06, 1.42, 1.71, 1.96; .32, .50, .83, 1.08, 1.15 for Na, K and Cl respectively. Actual concentrations were used in statistical analysis.

[2] CAD = meq (Na + K - Cl)/100g diet DM.

[3] Na and Cl concentrations intentionally were not formulated at their very low and very high concentrations, respectively.

Next factorial points (treatments 1 through 8) were selected at concentrations below and above the center point (Na ± .2%, K ± .3%, Cl ± .3%). These eight combinations of Na, K and Cl comprised the eight vertices of Figure 3-1. The figure is drawn in the metric of what are called coded or design variables. These were defined as: x_1 = (Na - .55)/.2, x_2 = (K - 1.4)/.3, x_3 = (Cl - .8)/.3. Note that the concentrations for the factorial treatments were determined by setting the values of x_1, x_2 and x_3 equal to +1 and -1 and then solving for Na, K and Cl. Also note that the coded value for the center point equaled 0. Once the center point treatment and eight factorial treatments were selected, axial points (treatments 9 through 14) were added to augment the factorial design so that a second order model could be fit and curvilinear effects could be studied. Axial points consisted of extreme values of Na, K and Cl that were the same distance from the center point as were the factorial points. The coded values of the axial points were ± 1.68. By using these coded values, factorial and axial points could be arranged spherically around the center point making the CCD rotatable, which is a desirable statistical property (Khuri and Cornell, 1987). When fitting axial points for each mineral (e.g., very low, .21%, and very high, .89%, concentrations of Na) the other two minerals were held at their middle (center point) concentration (e.g., 1.4% K and .8% Cl). Exact axial concentrations for all treatments (i.e., very low Na and very high Cl) were not formulated because of the potential deficiency or toxicity at these concentrations (Table 3-3). Consequently, responses at these concentrations were determined by extrapolation.

Ingredients used to alter concentrations of Na, K and Cl in different treatments included NaCl, KCl, $CaCl_2$, Na_2CO_3, and K_2CO_3. Carbonate salts were used in place of bicarbonate salts because they contributed more Na and K, and less organic anion (i.e., dietary buffer). When a formulation choice existed, NaCl and KCl were chosen over $CaCl_2$ due to the potential toxicity of $CaCl_2$ (Tucker et al, 1988b). Calcium carbonate was used to maintain constant Ca concentration; SiO_2 (washed sand) was used to maintain constant ash concentration in all dietary treatments. Diets were formulated to be equal in all other nutrients and energy.

The center point (treatment 15) had a total of 24 cow-period replications, whereas the other 14 treatments had a total of 12 cow-period replications. With the exception of treatment 15 (which had three cows remain on that treatment throughout the entire experiment), every treatment occurred with every other treatment within the same cow-period sequence at least twice but no more than three times. No treatment followed another treatment more than once and treatments were assigned to each cow only once throughout the entire experiment. A total of 192 cow-period observations were attainable for each dependent variable.

Sample Collection and Analysis

Intake and milk production data for statistical analyses were collected during the last 2 wk of each period. Two milk samples from each cow were collected at each milking (twice daily) during the last 3 d of each period. One sample preserved with $K_2Cr_2O_7$ was analyzed for

fat and protein concentration by DHIA Testing Laboratory (Raleigh, NC). The unpreserved milk sample was frozen (-10 °C) for later mineral analysis. Milk fat composition from the last six milkings (weighted to corresponding milk weight) was used to calculate 3.5% FCM yield. Body weights were recorded on three afternoons immediately prior to the start of the experiment and after the p.m. milking on d 25, 26 and 27 of each experimental period.

Corn silage was sampled three times weekly for DM. Amounts of corn silage fed were adjusted as needed to maintain desired DM proportions of ration components. Corn silage and batch mixes (which included 5.5% cottonseed hulls) were sampled, dried for 48 h at 55 °C, ground through a 2 mm screen and frozen at -10 °C for later analyses. Samples of corn silage and concentrate were analyzed by commercial laboratory (Northeast DHIA Forage Testing Lab, Ithaca NY) for DM, CP, ADF, Ca, P, Mg, S, Fe, Zn, Cu, and Mn. Energy concentration (NE_L) was calculated from chemical analysis. Concentrate and corn silage Na, K and Cl were determined from analyses of composite samples in the Dairy Nutrition Laboratory at the University of Florida. Thawed feed samples were dried (100 °C for 24 h) and ashed (550 °C for 4 h). Ash was dissolved in 3N HCl solution, diluted with deionized water and analyzed via atomic absorption spectrophotometry (Model 5000, Perkin Elmer, Inc., Norwalk, CT). Feed samples for Cl analysis were dissolved in 25 ml of a .4N HNO_3 40% glacial acetic acid solution, shaken vigorously for 1 h and centrifuged at 12,000 x g for 10 min. Supernatant was harvested and analyzed for Cl by coulometric titration (Model 4-2500, Haake Buchler Instruments, Inc., Saddlebrook, NJ; Cotlove, 1963).

On the morning of d 28 of each period, immediately following milking but prior to feeding, blood samples were collected from the jugular vein of each cow. A 5 ml sample was collected anaerobically into plastic syringes coated with ammonium heparin (200 U/ml). This blood sample was kept on ice and analyzed for blood pH and partial pressure of CO_2 (pCO_2) within 2 h of collection (Model 1304 pH/blood gas analyzer, Instrumentation Labs, Lexington, MA). Blood bicarbonate (HCO_3) was calculated from blood pH and pCO_2 according to the Henderson-Hasselbach equation (Kleinman and Lorenz, 1989).

Another 25 ml blood sample was collected from each cow in 14-ml plastic syringes coated with ammonium heparin (20 U/ml) and decanted into two 14-ml plastic tubes. One tube of blood was centrifuged (2500 x g) for 20 min. Plasma was harvested, transferred to 7-ml plastic tubes, kept on ice for 4 h and then frozen (-10 °C) for later mineral analyses. The other tube of whole blood was kept on ice for 4 h and then frozen (-10 °C) for later mineral analyses. Plasma concentrations of Ca, Mg, K and Na were analyzed after thawed samples were deproteinized with 10% TCA, vortexed, centrifuged at 2500 x g for 10 min, and diluted with deionized water. Milk samples were thawed and pooled within cow-period prior to analysis. Whole blood and milk concentrations of Ca, Mg, K and Na were analyzed after wet digestion of 1 ml of sample in 3 ml of concentrated HNO_3, heated in 30 ml glass tubes for 20 min, and diluted with deionized water. Atomic absorption analysis for plasma, whole blood and milk Ca, Mg, K and Na were analyzed utilizing the same method as for feeds. Whole blood and milk Cl were determined after acid-zinc sulfate protein precipitation (somogyi precipitate, Cotlove, 1963).

Plasma Cl concentration was determined by coulometric titration (Cotlove, 1963).

Original plans were to collect blood and urine from a subgroup of 24 cows, but due to a time delay in urine collection and the need for the rapid analysis of blood gasses, urine sampling was abandoned in favor of collecting additional blood samples. Therefore, 24 blood samples were collected in period one and 48 were collected in periods two through four.

Statistical Analysis

Least squares ANOVA general linear model (GLM) procedures of SAS (1985) were used. The analysis was conducted in two stages. In stage one, sources of variation included effects of cow, period, dietary treatment and residual error. Least squares means (LSMS) for treatments were calculated and then used as observations in stage two.

In stage two, treatment effects were partitioned into linear, quadratic and two-way interaction terms expressed in model form and used to fit a complete second order response surface. The mathematical model (or second-order polynomial) fitted was: $Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \epsilon$, where Y = dependent variable (or observed response variable), β_0 = constant (or intercept); β_1, β_2, β_3 are linear coefficients or parameters for Na, K and Cl; β_{11}, β_{22}, β_{33} are quadratic coefficients for Na, K and Cl; β_{12}, β_{13}, β_{23} are crossproduct or interaction coefficients; and ϵ = residual effect. For convenience, the chemical abbreviations (Na, K and Cl) were substituted for x_1, x_2, and x_3, respectively, in reported models.

The LSMS were analyzed by the fitting of these multiple regression equations using PROC REG (SAS, 1985). Higher order terms that did not attain a significance level of P = .15 were dropped from the model using the method of Maximum R^2 (SAS, 1985). This resulted in simpler, reduced model forms in all cases. The maximum R^2 method was used previously in a CCD used to study the effects of dietary Na, K, Cl on growing pigs (Golz and Crenshaw, 1990). To verify the utility of using LSMS instead of the individual cow-period observations, parameter estimates (for all response variables) from reduced models were compared with those that included all cow-period observations (with cow and period terms plus the reduced set of continuous linear, quadratic and two-way Na, K and Cl interaction terms). Parameter estimates were the same in both analyses (LSMS and cow-period models). Because probability values may change after model reduction, a moderate significance level ($P < .15$) was chosen to prevent removal of variables that might contribute to the predictive power. For several response variables, a significant effect ($P < .15$) in the LSMS model was not significant ($P > .15$) in the cow-period models. These effects were included in reduced models, nonetheless. Linear effects of Na, K and Cl variables always were included in each model (independent of level of probability). Probability values and standard errors of the coefficient estimates were generated from fitting the reduced model form in the cow-period models. Intercept and parameter estimates from LSMS-reduced models were used to generate response surfaces; R^2 values were from LSMS models.

If a reduced model contained a negative quadratic mineral term there was a concentration of that mineral within the experimental region that

maximized the response. The concentration that maximized a response was determined by setting the first partial derivative of the quadratic equation equal to zero and then solving. Concentrations of other minerals in the quadratic equation were set at their mean concentration for this calculation.

Results

Intake, Milk Yield, Milk Composition, and Body Weight Gain

Figures 3-2 through 3-9 present dry matter intake (DMI), 3.5% FCM yield, milk composition and body weight change response surface plots generated from reduced models. Surface plots were helpful to visualize the simultaneous change in concentrations of two minerals while holding the third mineral at its mean concentration. Yield of 3.5% FCM response was essentially equivalent to actual milk yield (MY) response (mean and SEM = 21.8 and .60; 21.5 and .67, respectively), therefore only the 3.5% FCM yield is presented. Listed in Table 3-4 are the probability (P) values (significance levels) associated with the coefficient estimates retained in the reduced models for all response variables. Only those effects with $P \leq .1$ were considered significant and are listed. When a two-way interaction was present, linear and curvilinear effects were not interpreted. When a curvilinear effect was present, linear effects were not interpreted.

Dry matter intake was affected by interactions between Na and K and between Na and Cl. Increasing dietary Na increased DMI only with low dietary K (Figure 3-2). Increasing dietary Na increased DMI only with

TABLE 3-4. Summary of P values for effects included in reduced models.[1]

Response	Na	K	Cl	Na^2	K^2	Cl^2	Na x K	Na x Cl	K x Cl	CAD	CAD^2	CAD^3
DMI	.28	.01	.02	...	...	...	.02	.01	...	<.01	.01	.05
MY	.09	.17	.66	...	...	.08	...	...	.09	.01	.02	...
FCM	.03	.15	.60	...	...	.07	...	...	.10	<.01	.01	...
FAT	.01	.12	.09	.08	.28	.06	.06	...	...	...	...	...
MP	.74	<.01	<.01	...	...	...	...	...	<.01	.09	.09	...
BWG	.17	<.01	<.01	...	...	...	...	...	<.01	.01	.03	.06
HCO3	.22	.34	.06	...	...	.11	...	...	...	.03	.09	...
BE	.05	.55	.14	.07	...	...	...	...	...	.08	...	...
pCO2	.07	.48	.10	...	...	...	...	.09	...	.03	.04	.06
PNa	.03	.16	.79	...	...	...	...	.03	.09	...	...	...
PK	<.01	.34	.63	.02	...	...	...	...	...	...	...	...
PCl	.04	.30	.01	...	...	.02	...	...	...	.02	...	...
PCa	.08	.73	.38	.09	...	...	...	...	...	...	...	...
WBNa	.61	.03	.46	...	...	...	...	...	...	...	...	...
WBK	.02	.17	.66	.03	.13	...	...	...	.17	...	...	...
WBCl	.79	.15	.11	...	.04	...	...	...	...	...	...	...
WBCa	.36	.06	.56	...	...	.07	...	...	.11	...	...	...
WBMg	.60	.76	.11	.19	...	...	...	.17	.10	...	...	...
MLK	.73	.02	.03	...	.02	...	...	...	...	...	...	...
MLCl	.18	.61	.03	...	...	.02	...	...	...	.07	...	...
MLCa	.44	.05	.07	.11	...	...	.25	...	.11	...	...	...
MLMg	<.01	.11	.09	...	...	...	...	...	...	...	...	...

[1]P values from Type III sums of squares.

[2]Quadratic effect.

[3]Cubic effect.

high dietary Cl (Figure 3-3). When dietary Na was increased and not Cl (or vice versa) DMI declined.

Yield of 3.5% FCM increased with increasing dietary Na (independent of dietary K and Cl concentration; Figure 3-4). Response to dietary K depended on the concentration of Cl (Figure 3-5). Although linear x quadratic interaction terms were not included in the initial mathematical models and thus were not tested statistically, there appeared to be an interaction between the quadratic Cl and linear K term. The shape of the quadratic response to Cl (Figures 3-5) was different for differing concentrations of K in the diet. Maximum 3.5% FCM yield response to dietary Cl depended upon the accompanying concentration of K.

Milk fat percentage was affected by a dietary Na x K interaction (Table 3-4) but this effect was overshadowed by the quadratic effects of Na, K, and Cl. Because the MF regression included all three (Na, K and Cl) quadratic terms whose coefficients were negative, it was conjectured that a maximum MF value existed within the experimental region. Figures 3-6 and 3-7 seem to support this conjecture. In fact, an estimated maximum MF value of 3.6% was discovered with .60% Na, 1.34% K and .69% Cl. This concentration of K may not have been optimal because the quadratic K term had a P value of .28 in the reduced model (Table 3-4). Had the K^2 term been removed, the Na x K effect likely would have been more influential, which in turn would have affected the optimal Na concentration.

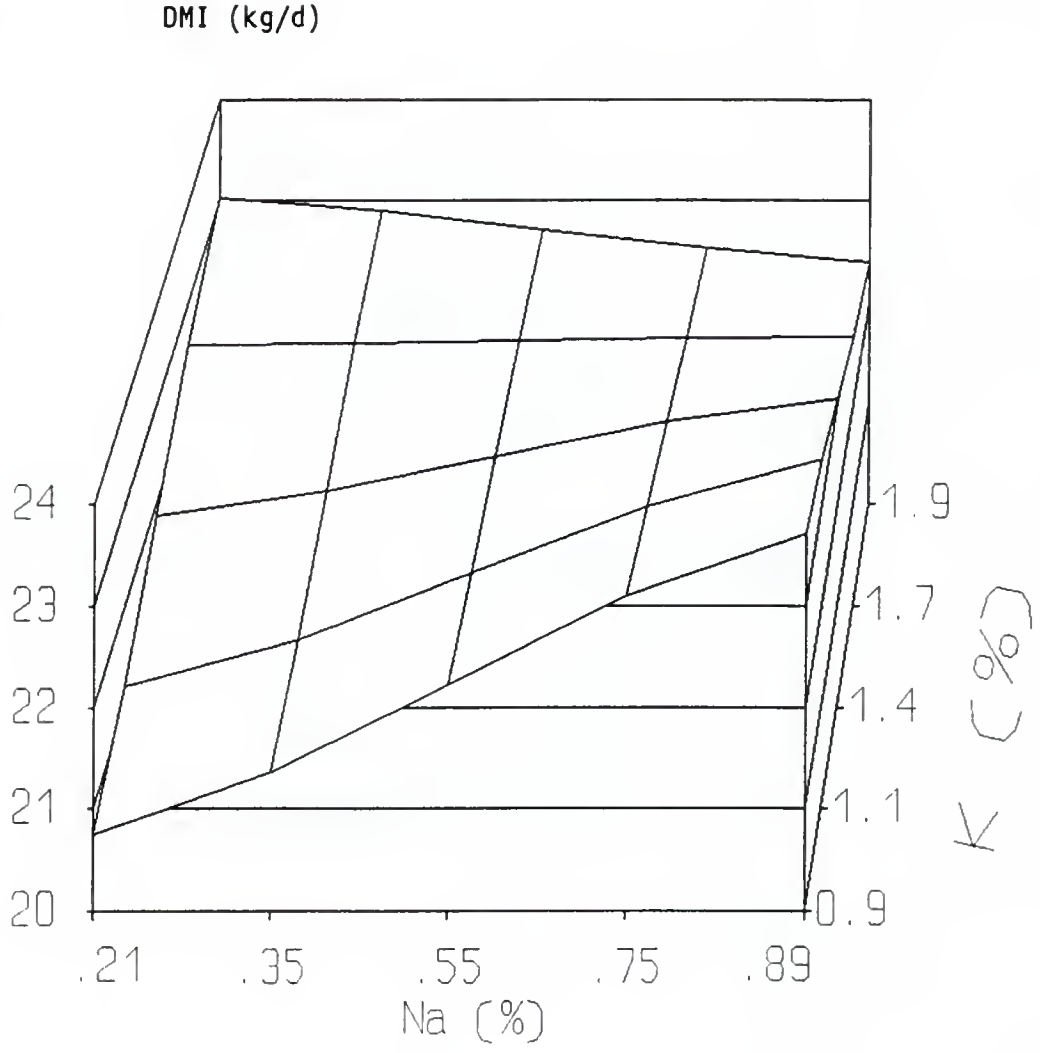

Figure 3-2. Response surface for DMI plotted against dietary Na and K with Cl fixed at .8%. Reduced model with SE for each coefficient in parentheses: DMI = 19.51 + 3.95 (3.56) Na + 3.39 (1.21) K - 3.40 (1.37) Cl - 5.28 (2.12) Na x K + 6.43 (2.38) Na x Cl. R^2 = .55. Mean and SEM for DMI = 22.5 and .38 kg/d

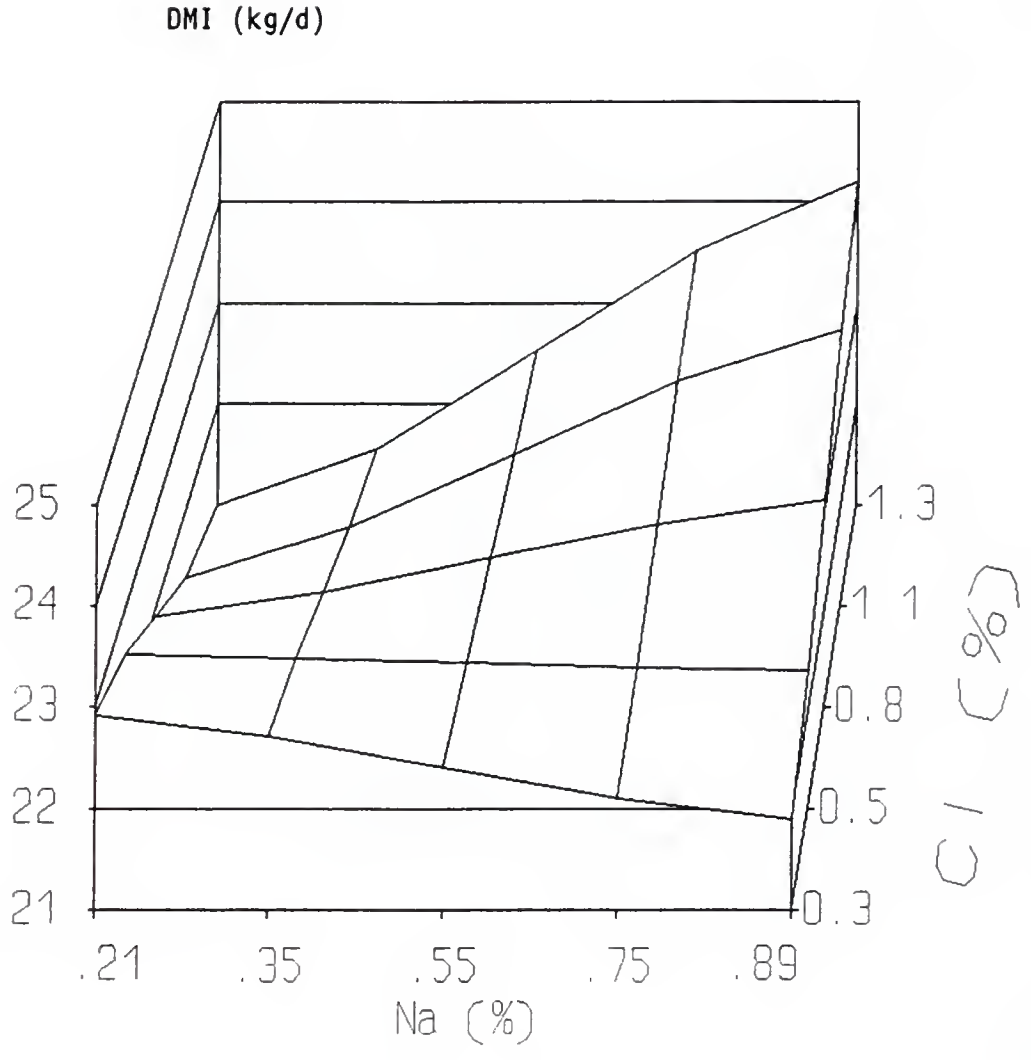

Figure 3-3. Response surface for DMI plotted against dietary Na and Cl with K fixed at 1.4%. Reduced model with SE for each coefficient in parentheses is: DMI = 19.51 + 3.95 (3.56) Na + 3.39 (1.21) K - 3.40 (1.37) Cl - 5.28 (2.12) Na x K + 6.43 (2.38) Na x Cl. R^2 = .55. Mean and SEM for DMI = 22.5 and .38 kg/d.

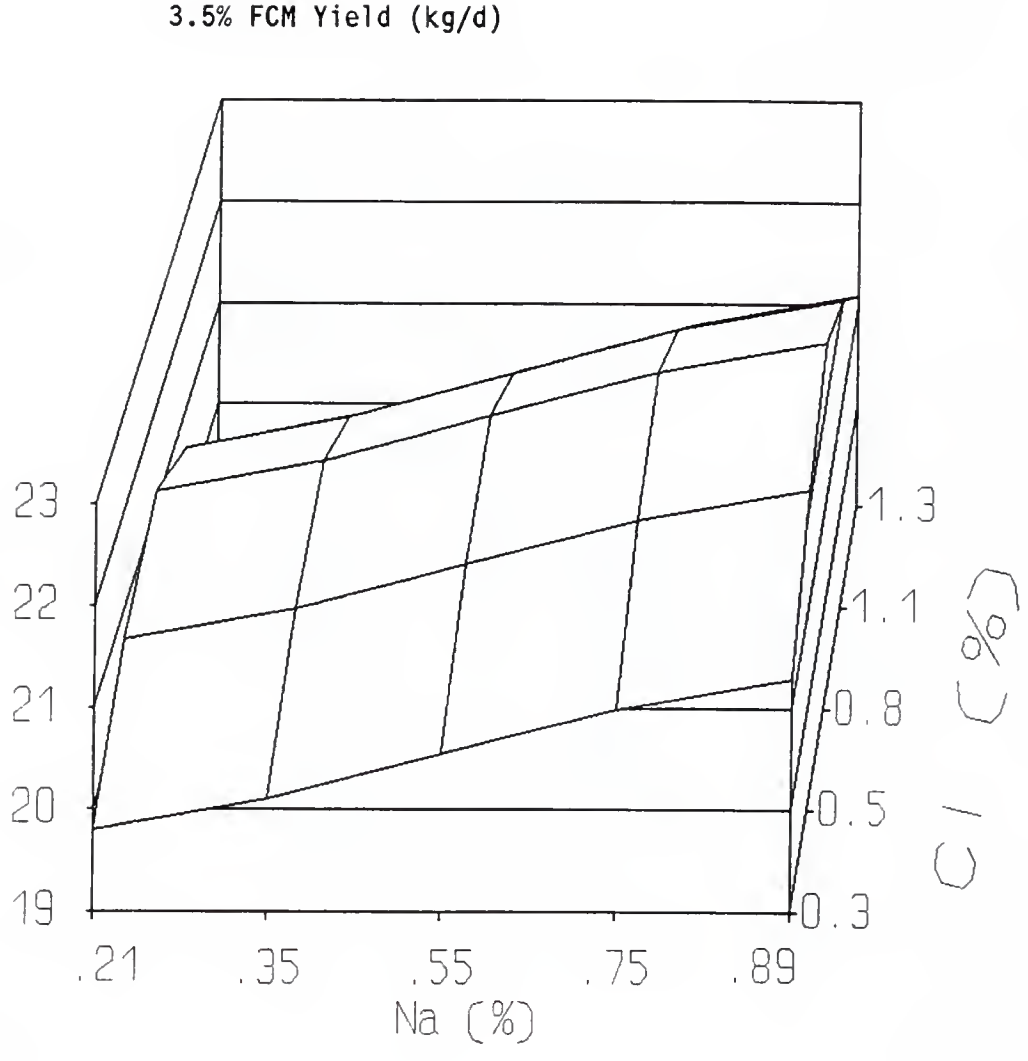

Figure 3-4. Response surface for 3.5% FCM (3.5% FCM) yield plotted against dietary Na and Cl with K fixed at 1.4%. Reduced model with SE for each coefficient in parentheses: 3.5% FCM yield = 21.41 + 2.19 (.97) Na - 3.02 (2.09) K + 2.36 (5.27) Cl - 5.68 (± 3.04) Cl^2 + 4.09 (2.51) K x Cl. R^2 = .64. Mean and SEM for 3.5% FCM = 21.5 and .67 kg/d.

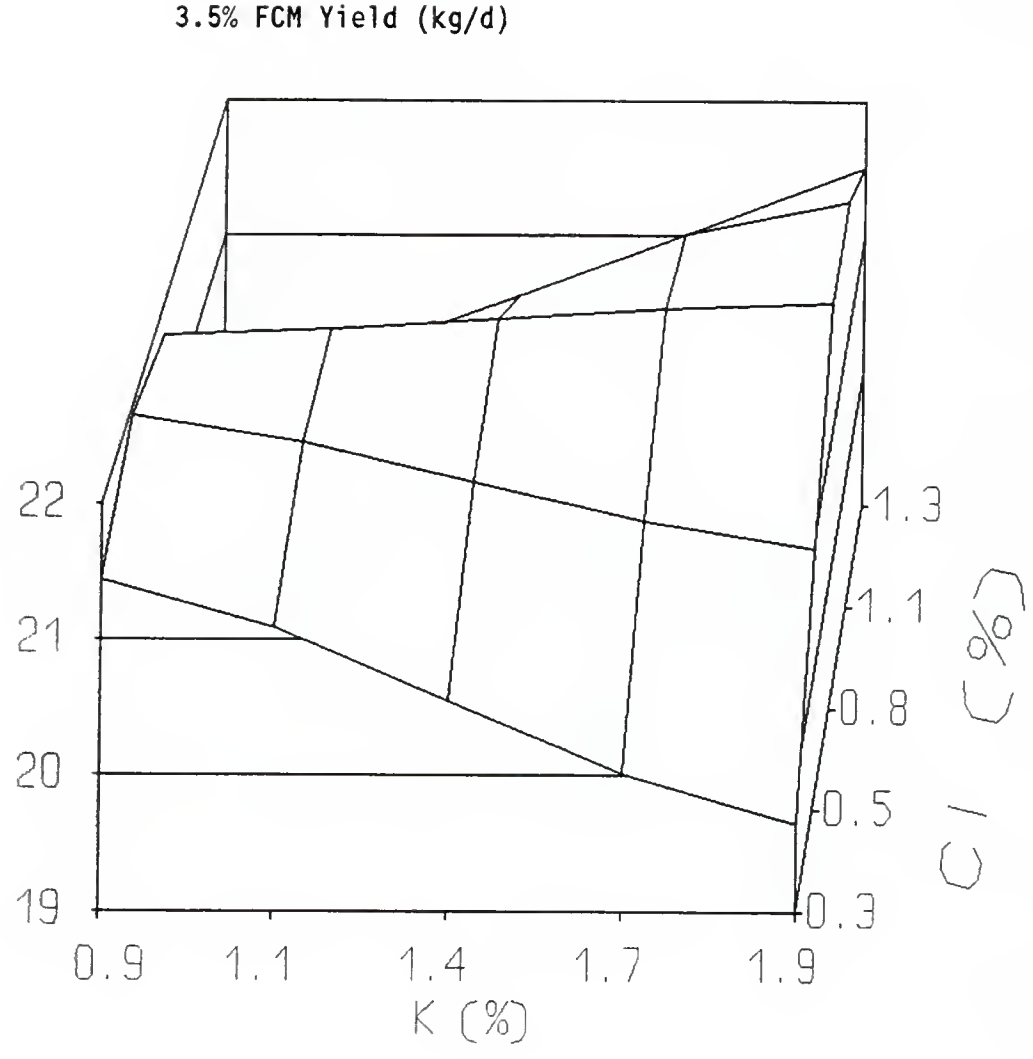

Figure 3-5. Response surface for 3.5% FCM (3.5% FCM) yield plotted against dietary K and Cl with Na fixed at .55%. Reduced model with SE for each coefficient in parentheses: 3.5% FCM yield = 21.41 + 2.19 (.97) Na - 3.02 (2.09) K + 2.36 (5.27) Cl - 5.68 (± 3.04) Cl^2 + 4.09 (2.51) K x Cl. R^2 = .64. Mean and SEM for 3.5% FCM = 21.5 and .67 kg/d.

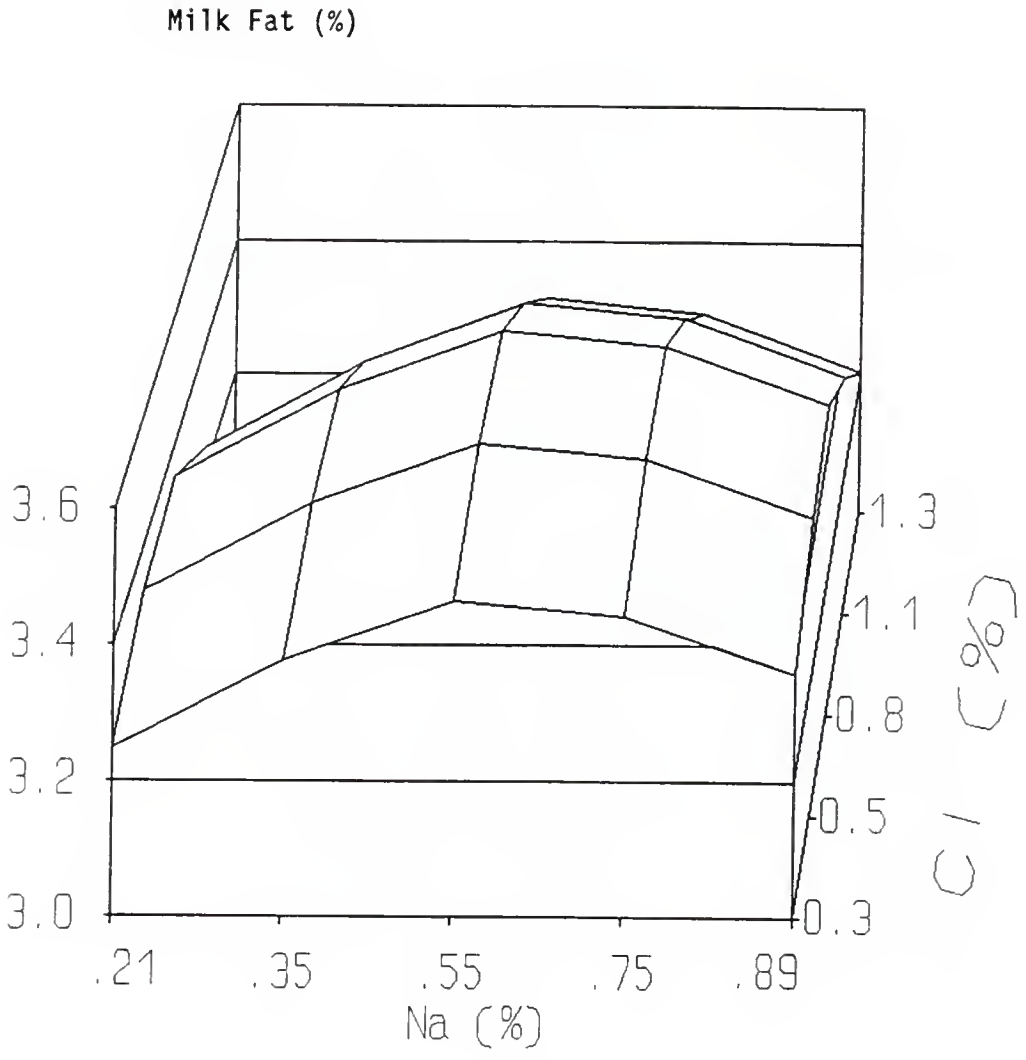

Figure 3-6. Response surface for milk fat percentage (MF) plotted against dietary Na and Cl with K fixed at 1.4%. Reduced model with SE for each coefficient in parentheses: MF = 1.74 + 2.71 (1.03) Na + 1.04 (.67) K + .97 (.56) Cl - 1.40 (.80) Na^2 - .24 (.23) K^2 - .70 (.37) Cl^2 - .72 (.37) Na x K. R^2 = .87. Mean and SEM for MF = 3.45 and .07 %.

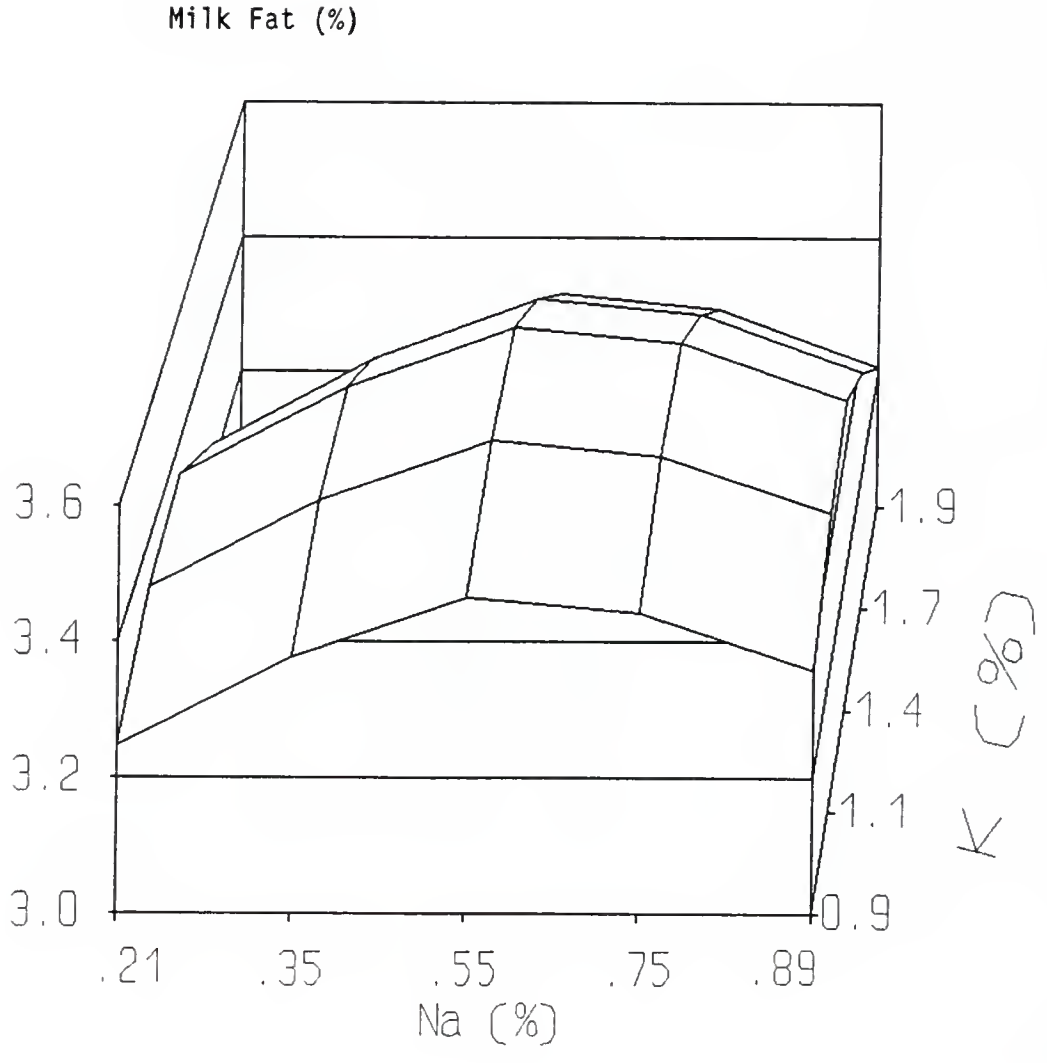

Figure 3-7. Response surface for milk fat percentage (MF) plotted against dietary Na and K with Cl fixed at .8%. Reduced model with SE for each coefficient in parentheses is: $MF = 1.74 + 2.71\ (1.03)\ Na + 1.04\ (.67)\ K + .97\ (.56)\ Cl - 1.40\ (.80)\ Na^2 - .24\ (.23)\ K^2 - .70\ (.37)\ Cl^2 - .72\ (.37)\ Na \times K$. $R^2 = .87$. Mean and SEM for MF = 3.45 and .07 %.

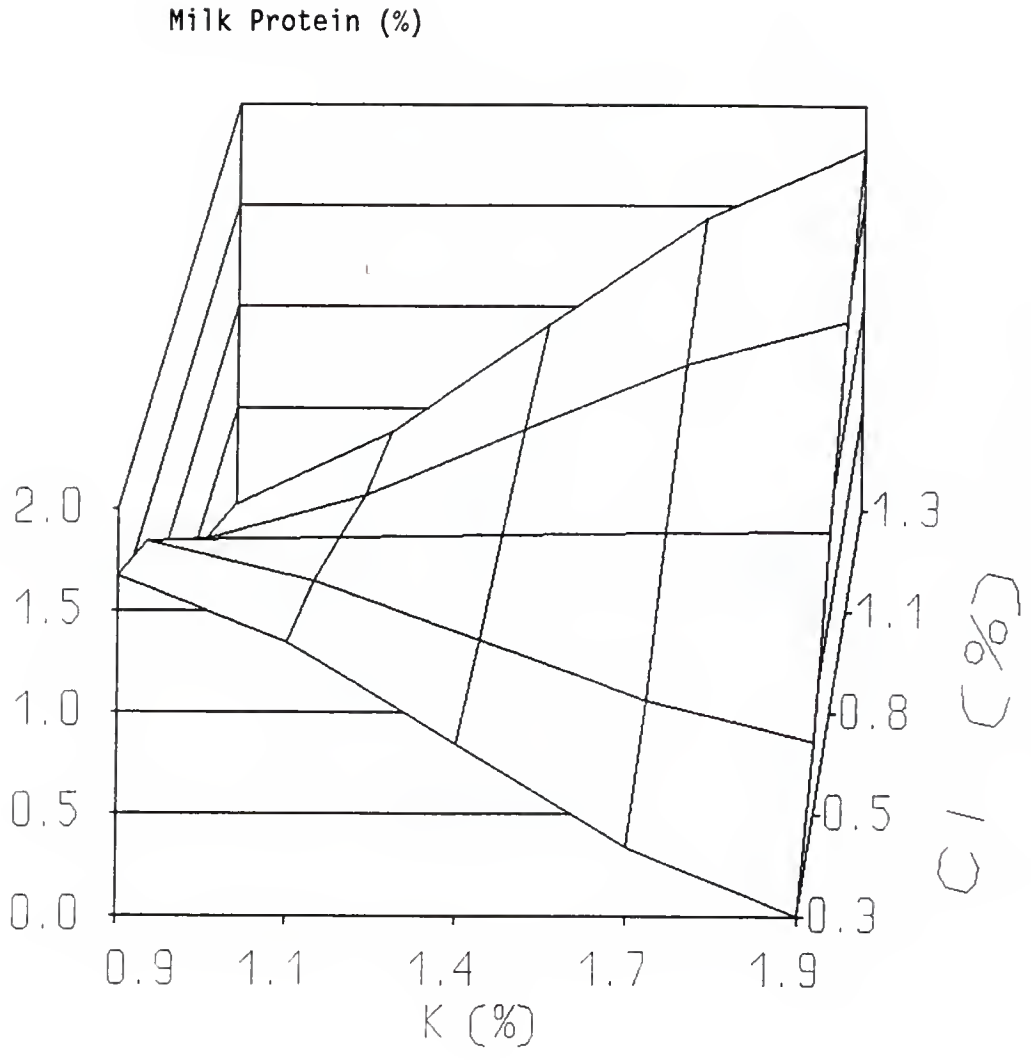

Figure 3-8. Response surface for milk protein percentage (MP) plotted against dietary K and Cl with Na fixed at .55%. Reduced model with SE for each coefficient in parentheses: MP = 3.56 - .02 (.07) Na - .50 (.15) K - .92 (.25) Cl + .65 (.18) K x Cl. R^2 = .53. Mean and SEM for MP = 2.83 and .05 %.

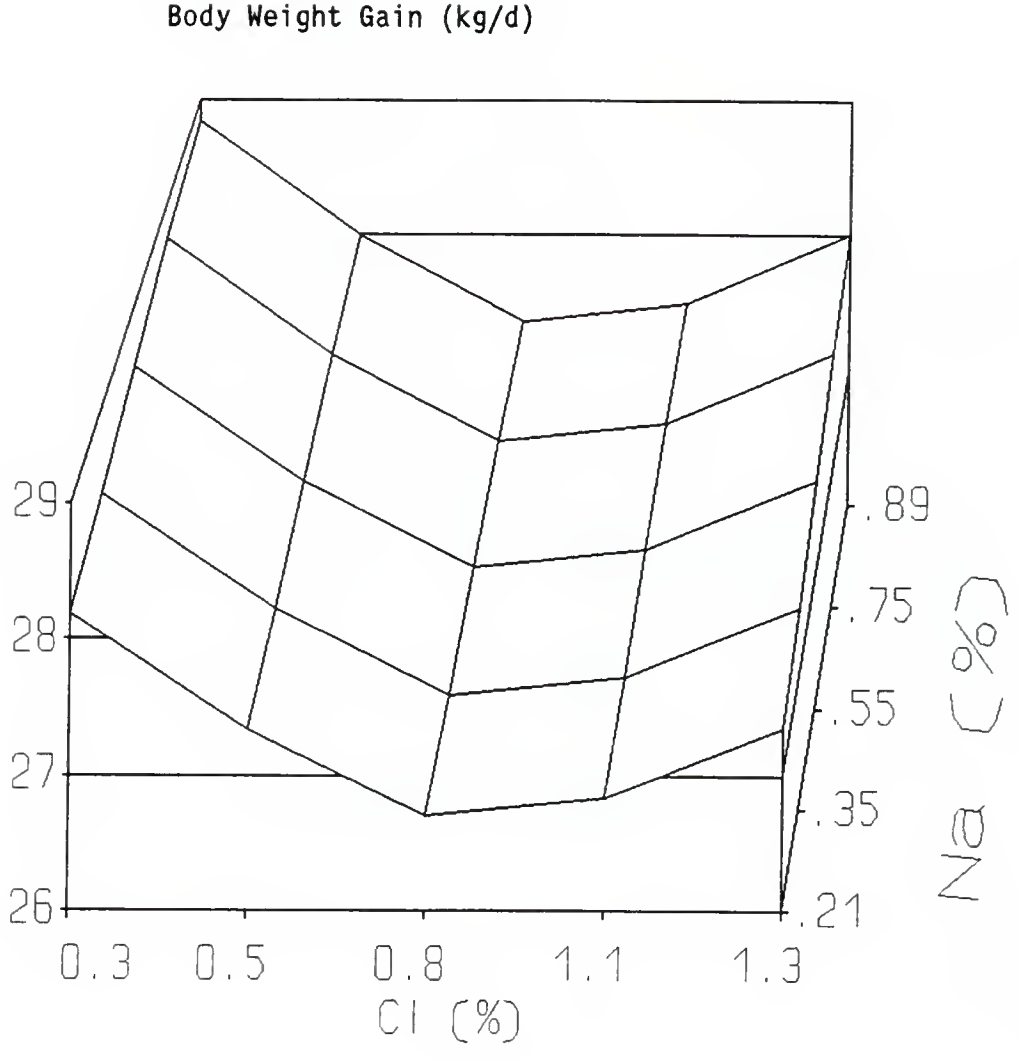

Figure 3-9. Response surface for body weight gain (BWG) plotted against dietary K and Cl with Na fixed at .55%. Reduced model with SE for each coefficient in parentheses: BWG = 4.25 + .63 (.46) Na - 2.68 (.98) K - 4.73 (1.67) Cl + 3.43 (1.18) K x Cl. R^2 = .38. Mean and SEM for BWG = .90 and .30 kg/d.

Acid-Base Status

Figures 3-10 through 3-12 show the response surface plots for blood, HCO_3^-, pCO_2, and base-excess. Values for pH were transformed to H^+ concentration [H^+] prior to generating LSMS. Least squares means for H^+ responses were then transformed back to pH for ease in interpretation, but pH data were not evaluated statistically (Murphy, 1982).

Although there appeared to be a quadratic effect of Cl on blood HCO_3^- (Figure 3-10), the coefficient estimates for the Cl^2 term was nonsignificant (P = .11). There was a significant negative linear effect of dietary Cl on blood HCO_3^- (Table 3-4). Blood pCO_2 was affected by Na x Cl interaction. Blood pCO_2 decreased with increasing concentrations of dietary Na when dietary Cl concentration was low, but increased with increasing dietary Na when dietary Cl concentration was high (Figure 3-11). Blood base excess (BE) responded quadratically to increasing dietary Na and was maximum at .60% Na (Figure 3-12). There was no effect of dietary Na, K or Cl on blood [H^+] or anion gap (calculated as meq [(Na + K) - (Cl + HCO_3)]/L in plasma) (P >.1) Mean and SEM for [H^+] and anion gap were 48.81 and 1.53; 5.72 and 3.29, respectively.

Mineral Metabolism

Figures 3-13 through 3-26 show the response surface plots for plasma; whole blood; and milk Na, K, Cl, Ca, and Mg responses. The only significant interactions were for plasma Na (Na x Cl and K x Cl; Figures 3-13 and 3-14) and whole blood Mg (K x Cl; Figure 3-22).

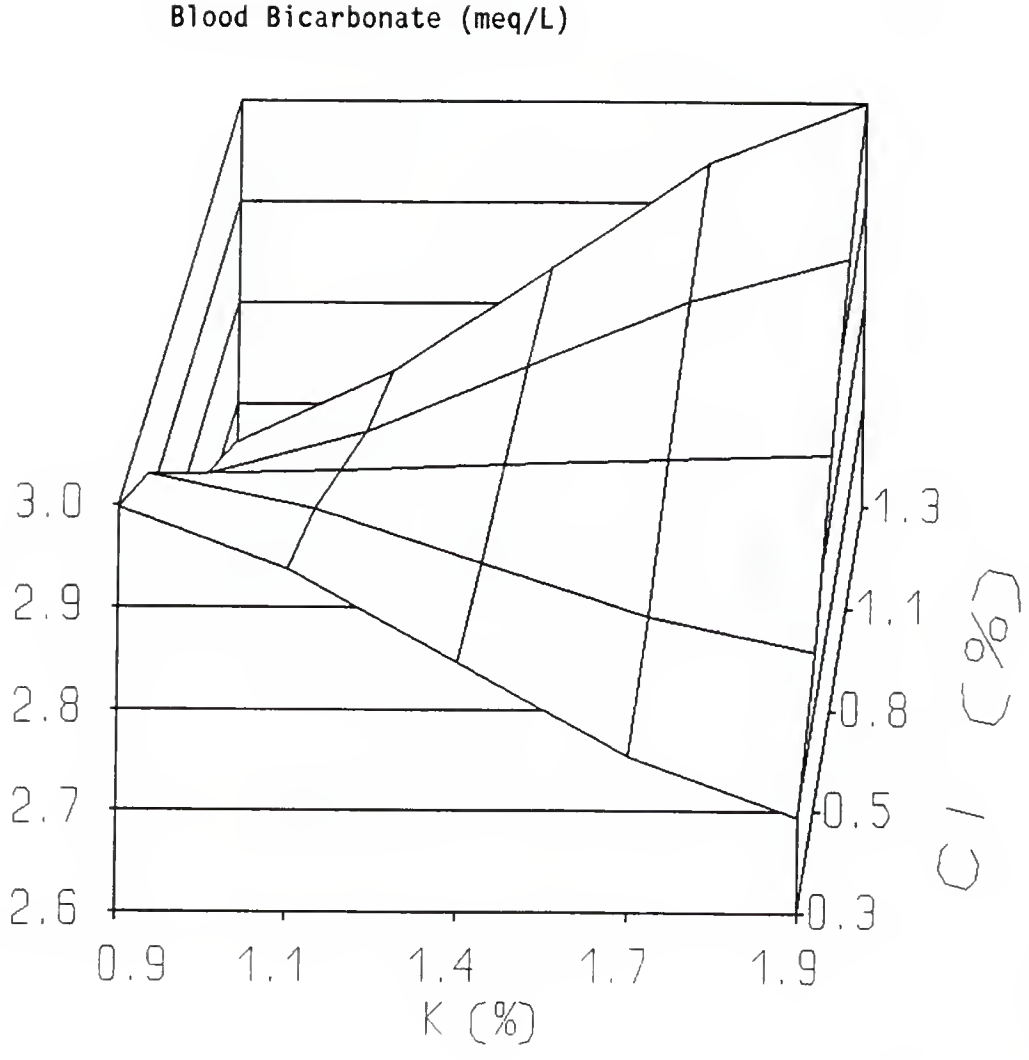

Figure 3-10. Response surface for blood bicarbonate (HCO_3^-) plotted against dietary Cl and Na with K fixed at 1.4%. Reduced model with SE for each coefficient in parentheses: HCO_3^- = 29.33 + .96 (.76) Na + .40 (.43) K - 7.63 (3.71) Cl + 4.25 (2.40) Cl^2. R^2 = .49. Mean and SEM for HCO_3^- = 27.33 and .51 meq/L.

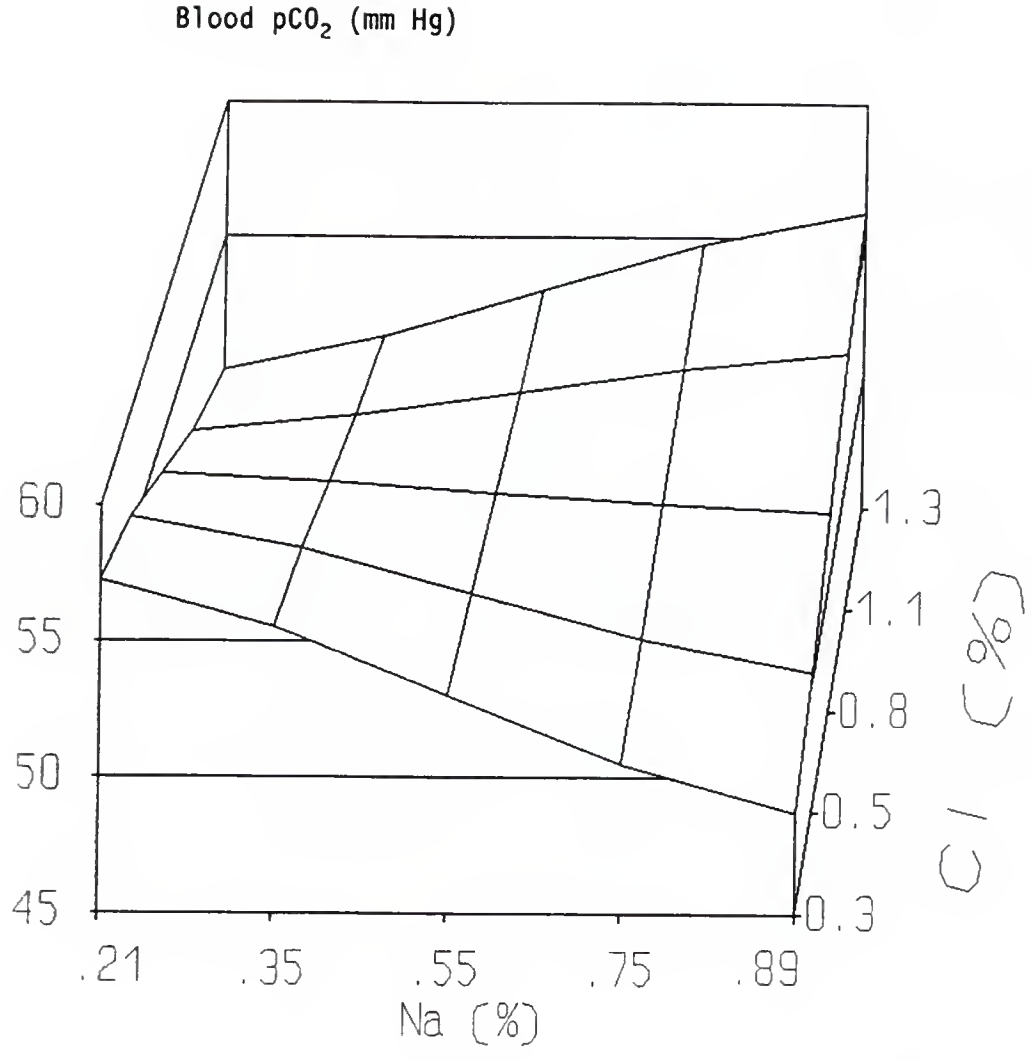

Figure 3-11. Response surface for blood pCO_2 plotted against dietary Na and Cl with K fixed at 1.4%. Reduced model with SE for each coefficient in parentheses: pCO_2 = 61.83 - 18.90 (10.49) Na + 1.12 (1.76) K - 11.62 (7.15) Cl + 21.15 (12.44) NaCl. R^2 = .34. Mean and SEM for pCO_2 = 53.00 and 2.14 mm Hg.

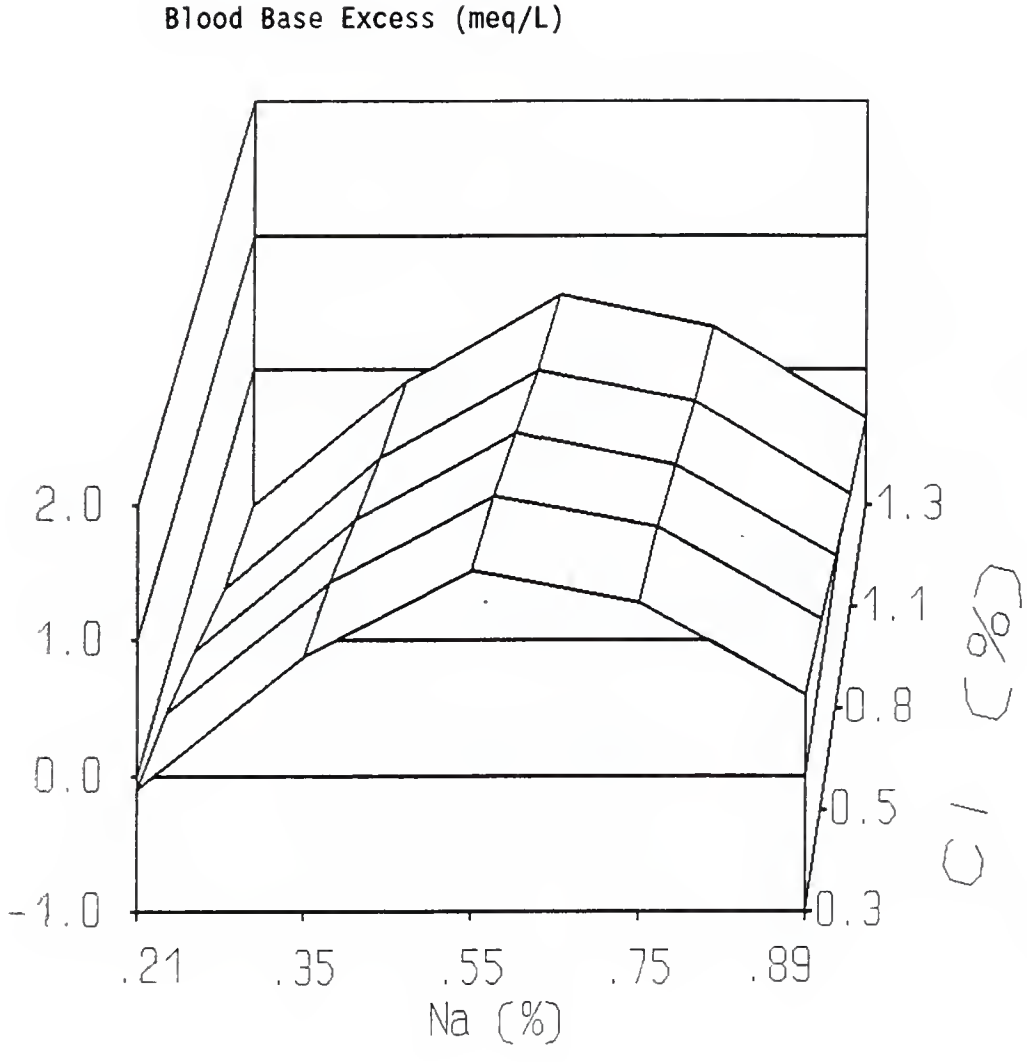

Figure 3-12. Response surface for blood base excess (BE) plotted against dietary Na and Cl with K fixed at 1.4%. Reduced model with SE for each coefficient in parentheses: BE = -2.41 + 13.03 (6.38) Na + .25 (.48) K - .96 (.56) Cl - 10.92 (5.75) Na^2. R^2 = .43. Mean and SEM for BE = .71 and .57 meq/L.

Plasma K (Figure 3-15), plasma Ca (Figure 3-17), and whole blood K (Figure 3-19) responded quadratically to increasing dietary Na. Whole blood Cl (Figure 3-20) and milk K (Figure 3-23) responded quadratically to increasing dietary K. Milk K was maximized with 1.42% K. Plasma Cl (Figure 3-16), whole blood Ca (Figure 3-21), and milk Cl (Figure 3-24) responded quadratically to increasing dietary Cl. Plasma Cl was maximal at .81% Cl.

There were few linear effects of dietary Na, K and Cl (independent from interaction effects) on mineral metabolism. Whole blood Na (Figure 3-18) decreased as dietary K increased. Milk Ca (Figure 3-25) decreased with increasing dietary Cl and milk Mg decreased with increasing dietary Na (Figure 3-26). Plasma Mg and milk Na were not affected by dietary Na, K or Cl ($P > .1$). Mean and SEM were 2.12 and .11; 23.17 and 1.18 for plasma Na and milk Mg, respectively.

Discussion

In this study response surface techniques were used to plot and interpret acid-base status, mineral metabolism and lactational performance responses to dietary Na, K and Cl. These methods were useful in helping to gain a cognizance of singular and interaction effects among dietary Na, K and Cl.

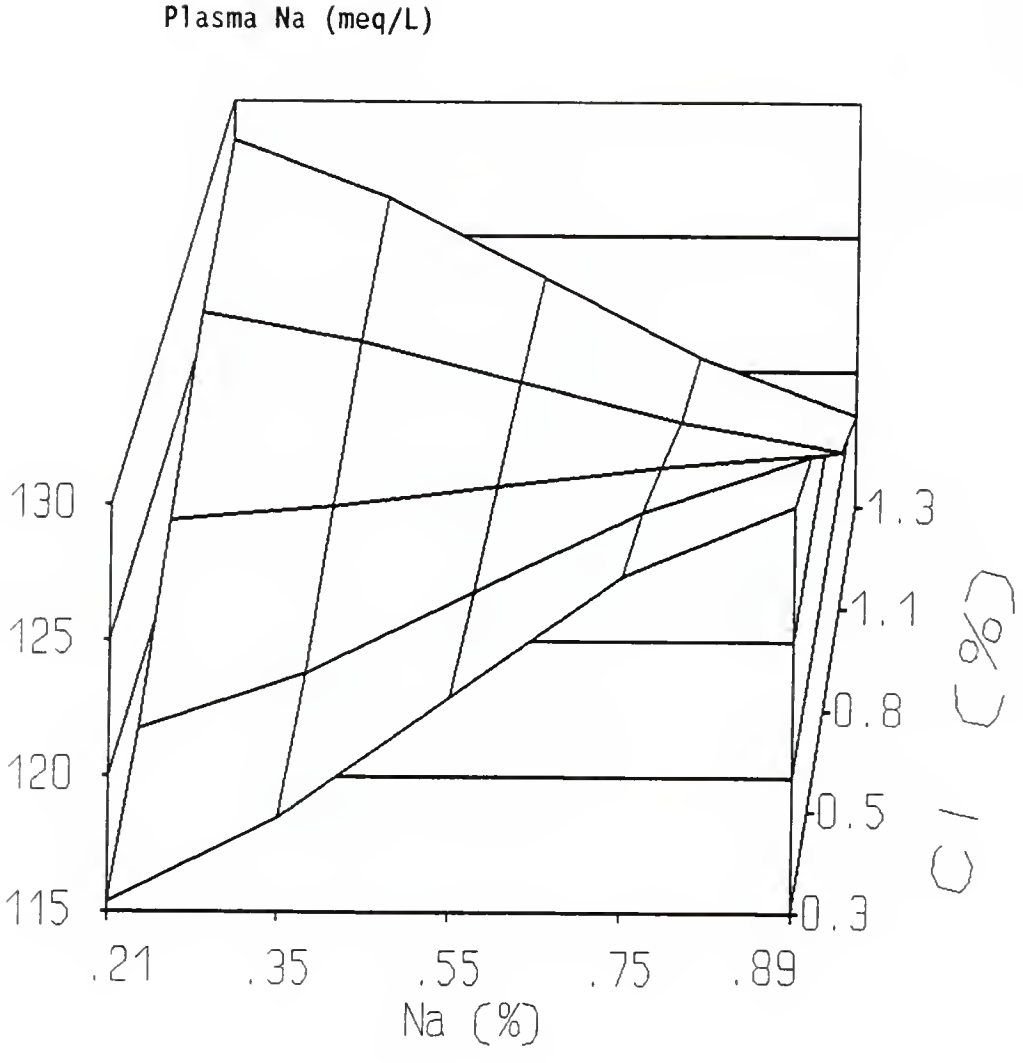

Figure 3-13. Response surface for plasma Na (PNa) plotted against dietary Na and Cl with K fixed at 1.4%. Reduced model with SE for each coefficient in parentheses: PNa = 124.89 + 33.41 (15.31) Na - 14.62 (9.57) K - 7.88 (18.90) Cl - 37.13 (18.15) Na x Cl + 20.58 (11.43) K x Cl. R^2 = .64. Mean and SEM for PNa = 123.27 and 3.14 meq/L.

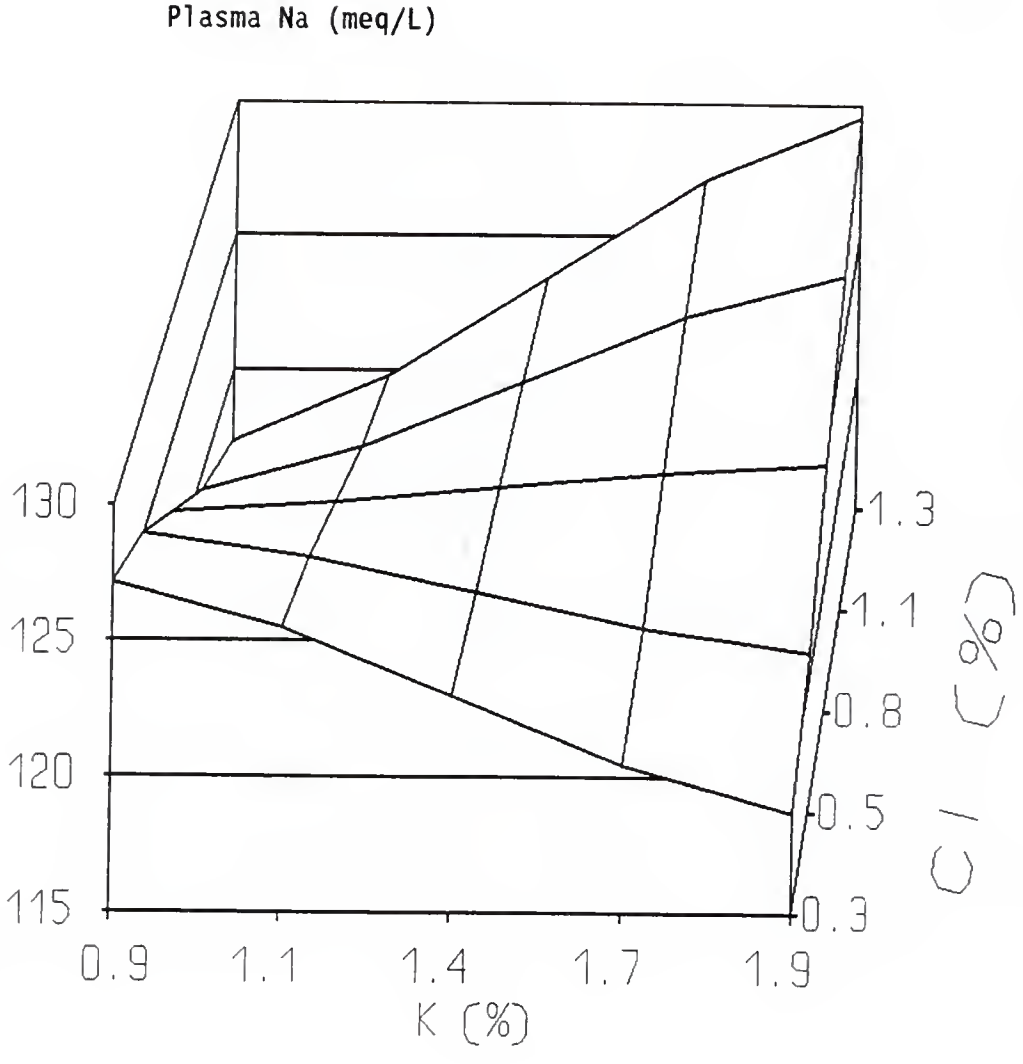

Figure 3-14. Response surface for plasma Na (PNa) plotted against dietary K and Cl with Na fixed at .55%. Reduced model with SE for each coefficient in parentheses is: PNa = 124.89 + 33.41 (15.31) Na - 14.62 (9.57) K - 7.88 (18.90) Cl - 37.13 (18.15) Na x Cl + 20.58 (11.43) K x Cl. R^2 = .64. Mean and SEM for PNa = 123.27 and 3.14 meq/L.

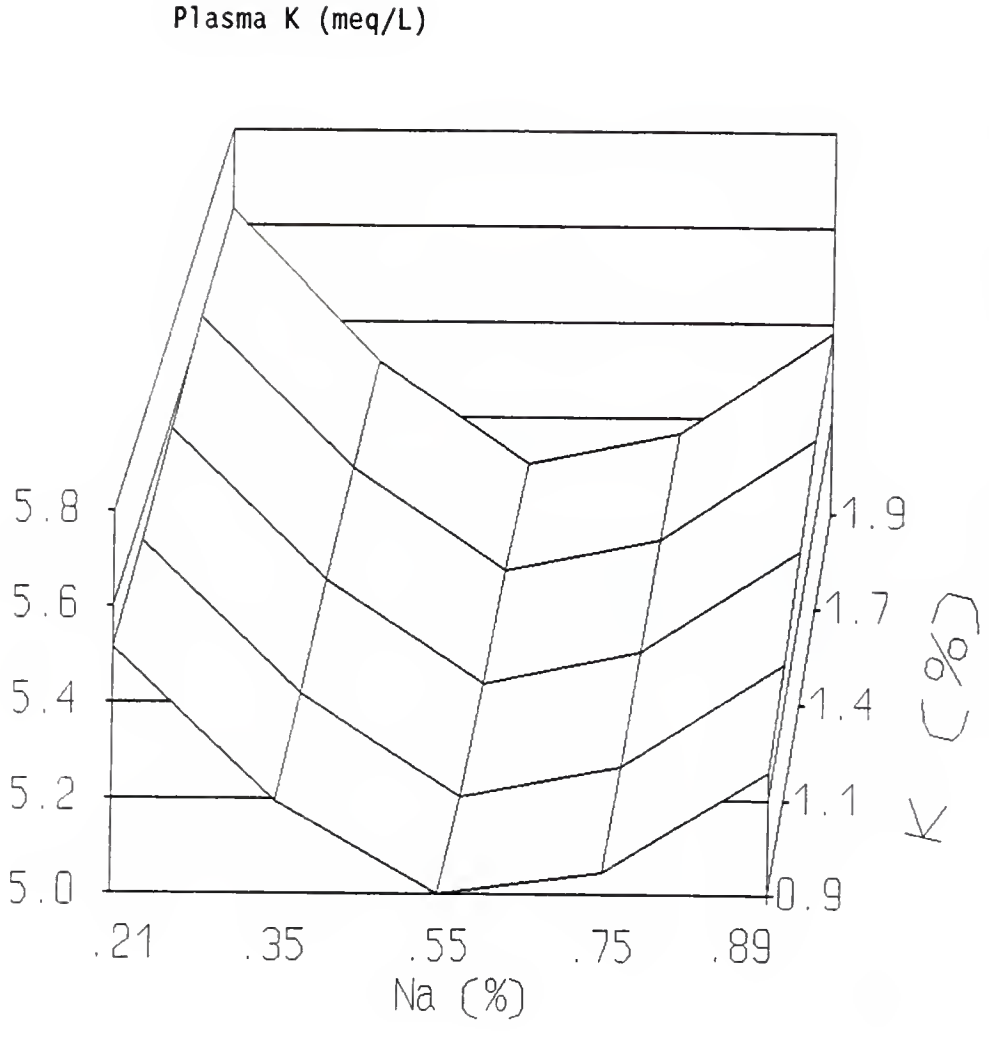

Figure 3-15. Response surface for plasma K (PK) plotted against dietary Na and K with Cl fixed at .8%. Reduced model with SE for each coefficient in parentheses: PK = 6.08 - 4.22 (1.65) Na + .12 (.12) K + .07 (.14) Cl + 3.50 (1.49) Na^2. R^2 = .47. Mean and SEM for PK = 5.15 and .15 meq/L.

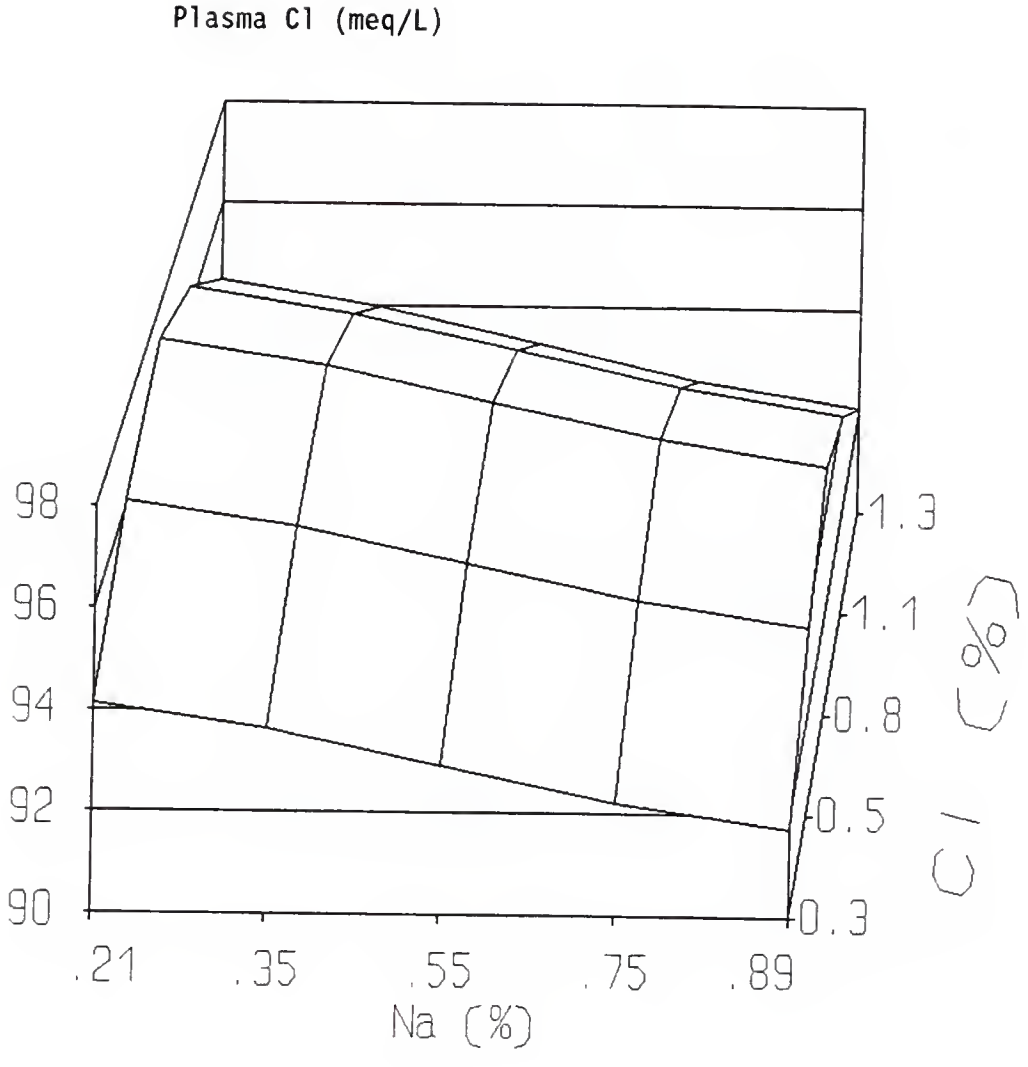

Figure 3-16. Response surface for plasma Cl (PCl) plotted against dietary Na and Cl with K fixed at 1.4%. Reduced model with SE for each coefficient in parentheses: PCl = 91.09 - 3.53 (1.60) Na - .72 (.90) K + 19.56 (7.80) Cl - 12.01 (5.04) Cl^2. R^2 = .50. Mean and SEM for PCl = 95.25 and 1.07 meq/L.

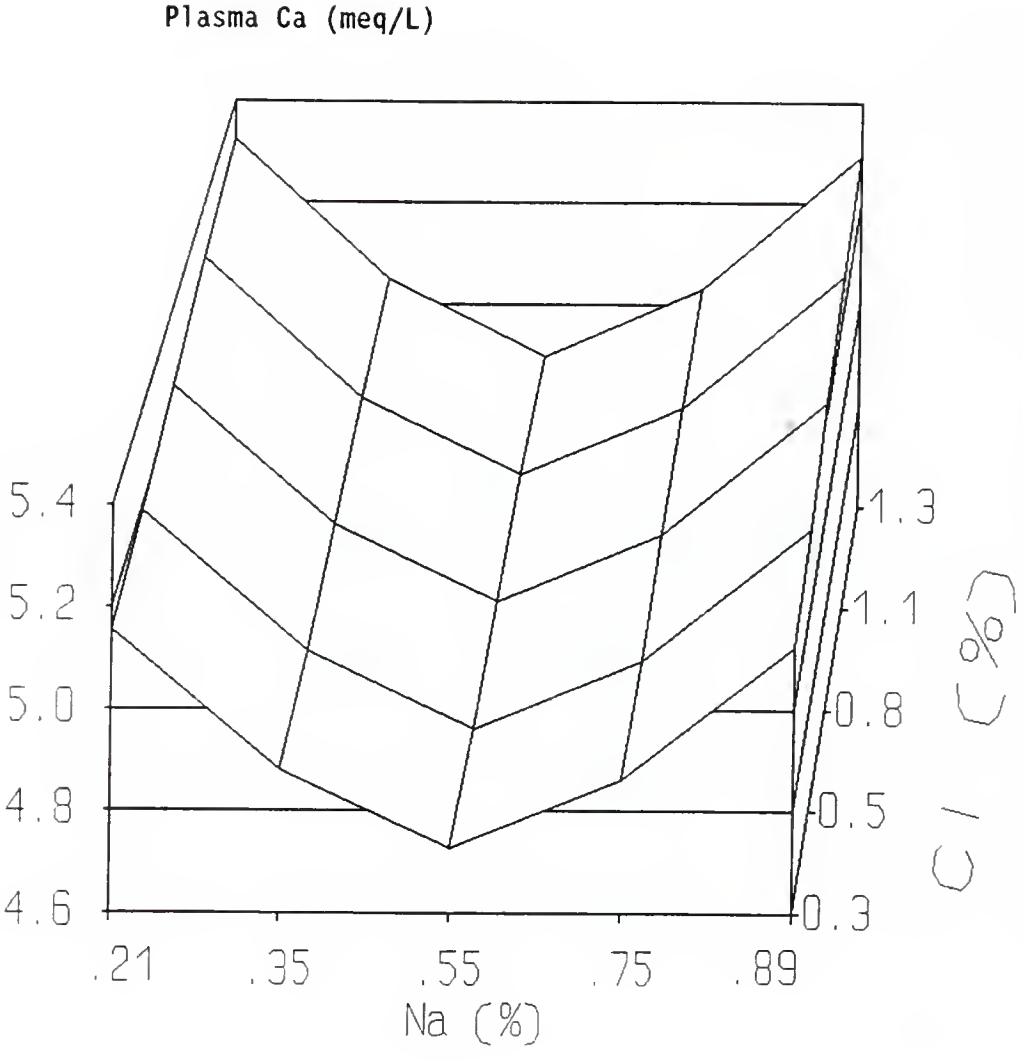

Figure 3-17. Response surface for plasma Ca (PCa) plotted against dietary Na and Cl with K fixed at 1.4%. Reduced model with SE for each coefficient in parentheses: PCa = 5.65 - 3.95 (2.21) Na + .09 (.17) K + .17 (.19) Cl + 3.55 (1.99) Na^2. R^2 = .30. Mean and SEM for PCa = 4.92 and .20 meq/L.

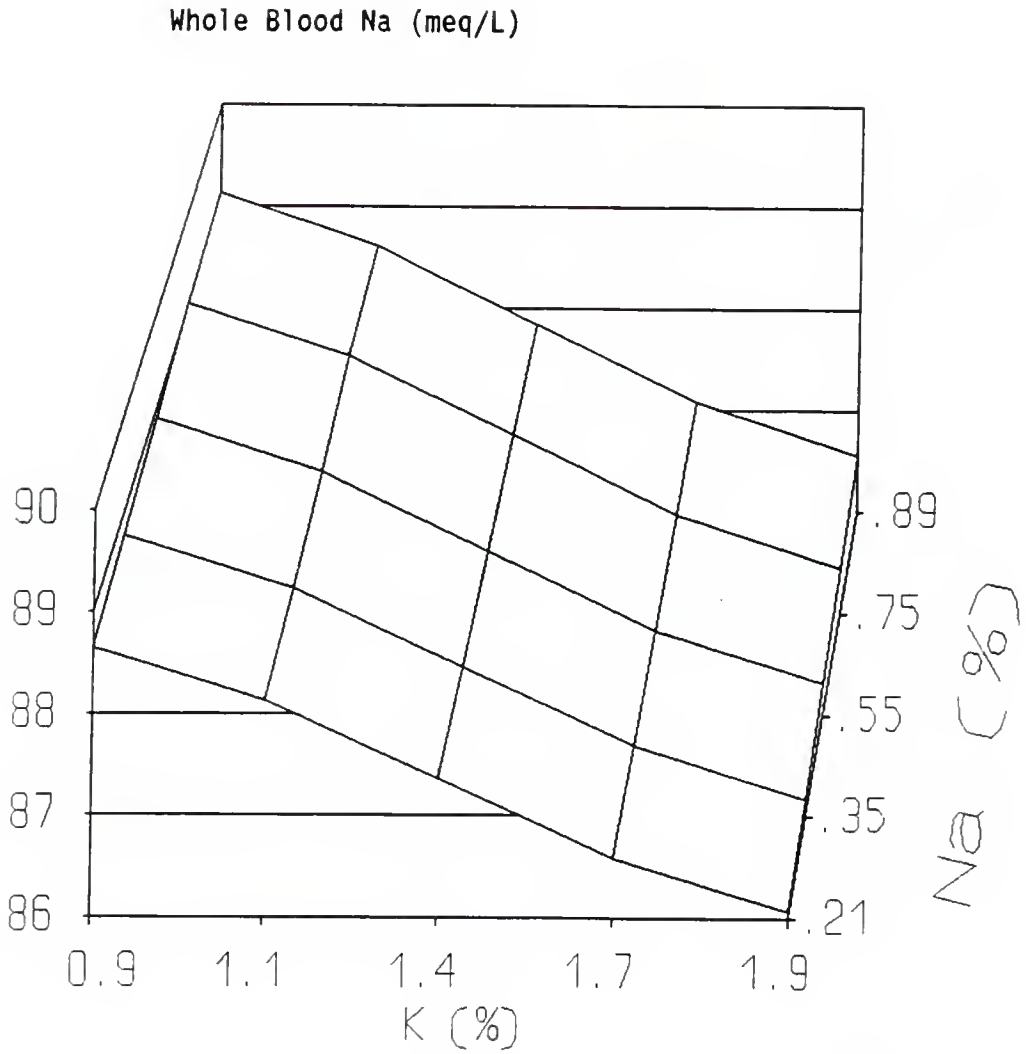

Figure 3-18. Response surface for whole blood Na (WBNa) plotted against dietary Na and K with Cl fixed at .8%. Reduced model with SE for each coefficient in parentheses: WBNa = 91.47 + .71 (2.05) Na - 2.58 (1.16) K - .81 (1.34) Cl. R^2 = .33. Mean and SEM for WBNa = 87.60 1.38 meq/L.

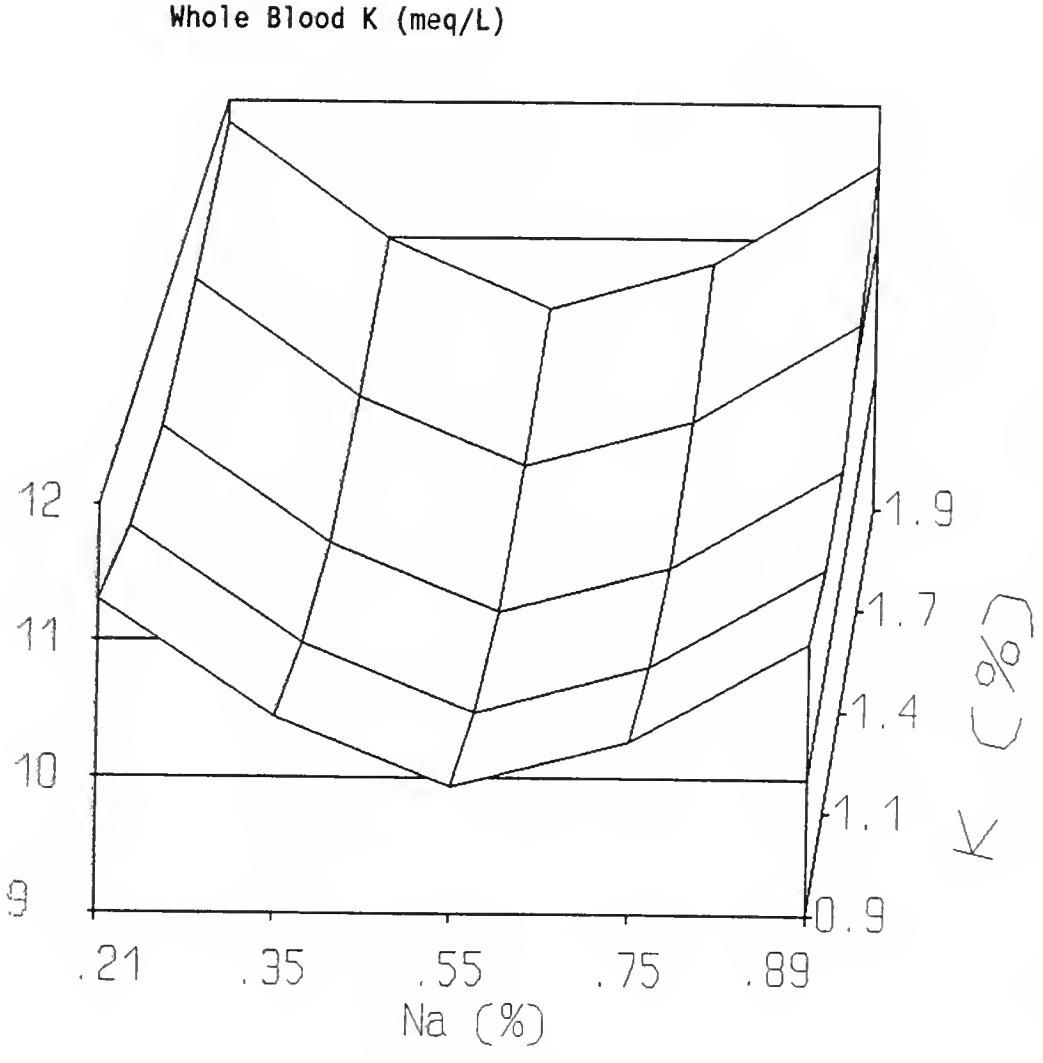

Figure 3-19. Response surface for whole blood K (WBK) plotted against dietary Na and K with Cl fixed at .8%. Reduced model with SE for each coefficient in parentheses: WBK = 16.03 - 12.01 (4.82) Na - 4.86 (3.31) K + .17 (.40) Cl + 10.54 (4.36) Na^2 + 1.93 (1.18) K^2. R^2 = .58. Mean and SEM for WBK = 10.23 and .42 meq/L.

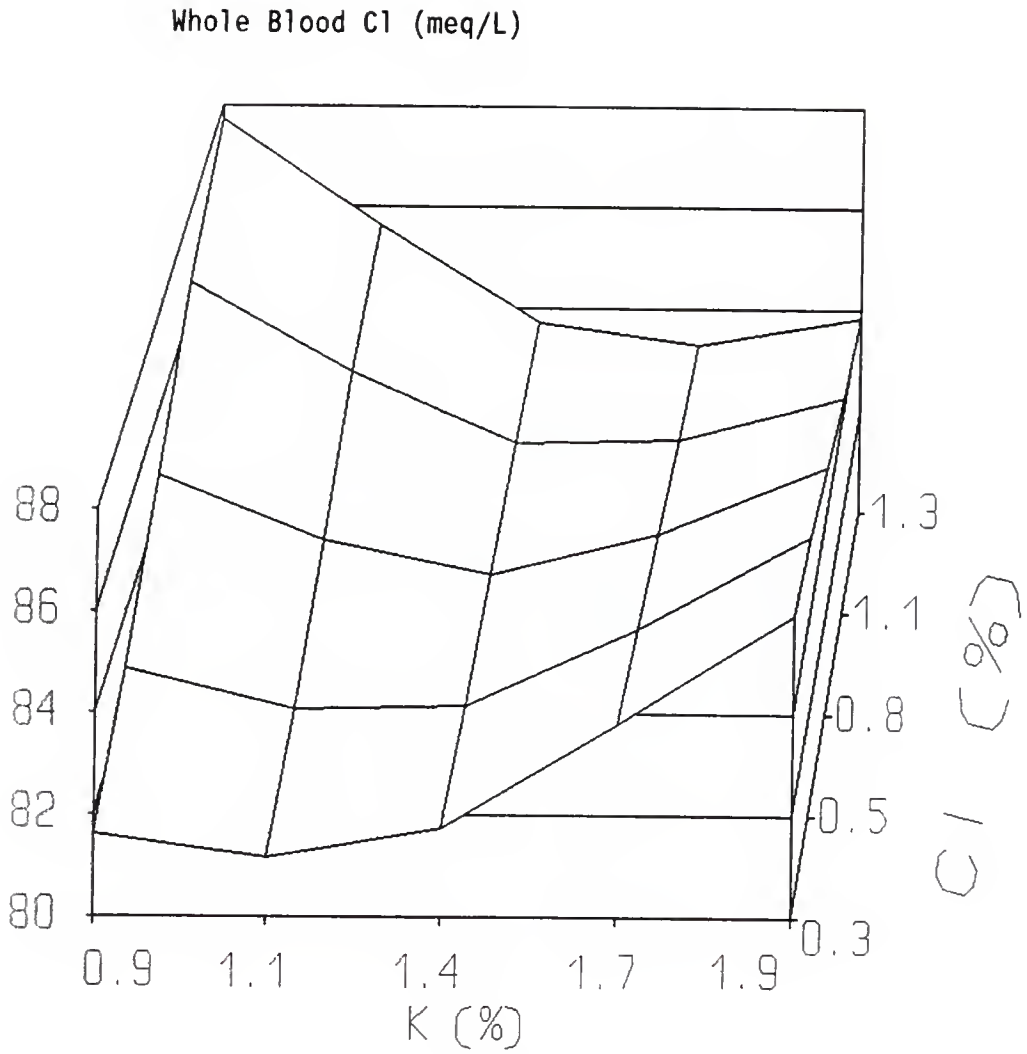

Figure 3-20. Response surface for whole blood Cl (WBCl) plotted against dietary K and Cl with Na fixed at .55%. Reduced model with SE for each coefficient in parentheses: WBCl = 88.09 - .70 (2.22) Na - 16.13 (12.57) K + 13.43 (7.94) Cl + 8.18 (4.21) Na^2 - 8.18 (5.55) K x Cl. R^2 = .53. Mean and SEM for WBCl = 83.50 and 1.51 meq/L.

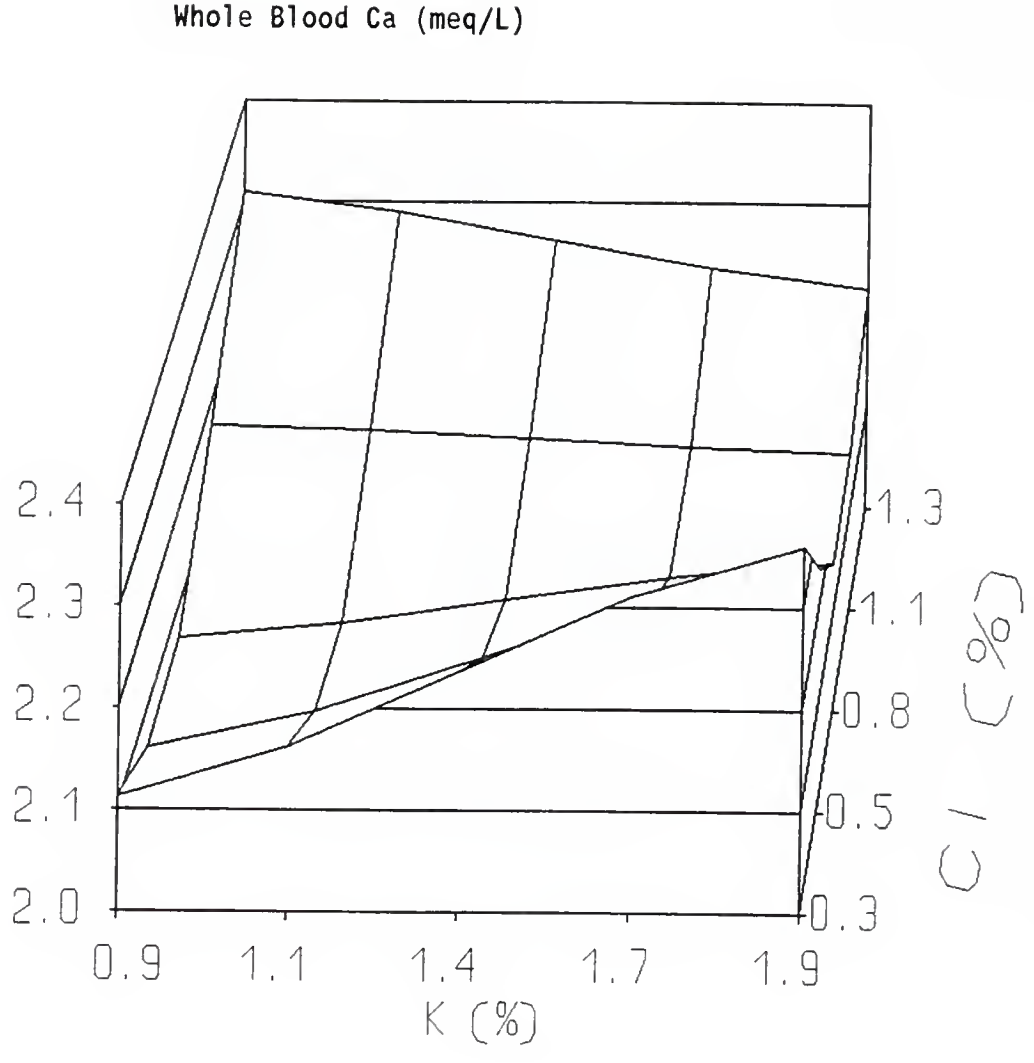

Figure 3-21. Response surface for whole blood Ca (WBCa) plotted against dietary K and Cl with Na fixed at .55%. Reduced model with SE for each coefficient in parentheses: WBCa = 2.00 - .07 (.09) Na + .35 (.18) K - .41 (.53) Cl + .57 (.27) Cl^2 - .34 (.22) K x Cl. R^2 = .57. Mean and SEM for WBCa = 2.15 and .06 meq/L.

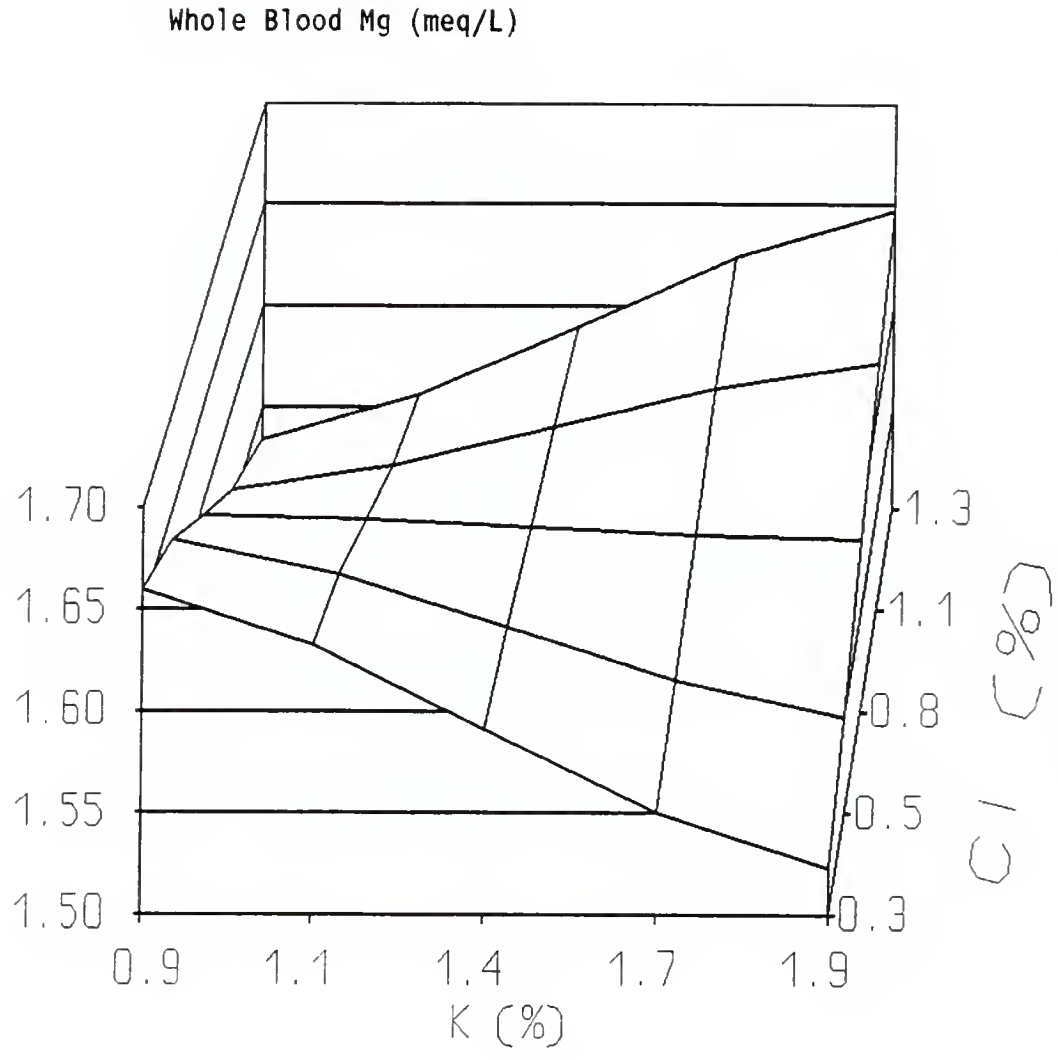

Figure 3-22. Response surface for whole blood Mg (WBMg) plotted against dietary K and Cl with Na fixed at .55%. Reduced model with SE for each coefficient in parentheses: WBMg = 1.89 - .30 (.54) Na - .06 (±.17) K - .35 (±.22) Cl + .55 (.41) Na^2 - .28 (.21) Na x K + .25 (.15) K x Cl. R^2 = .76. Mean and SEM for WBMg = 1.61 and .04 meq/L.

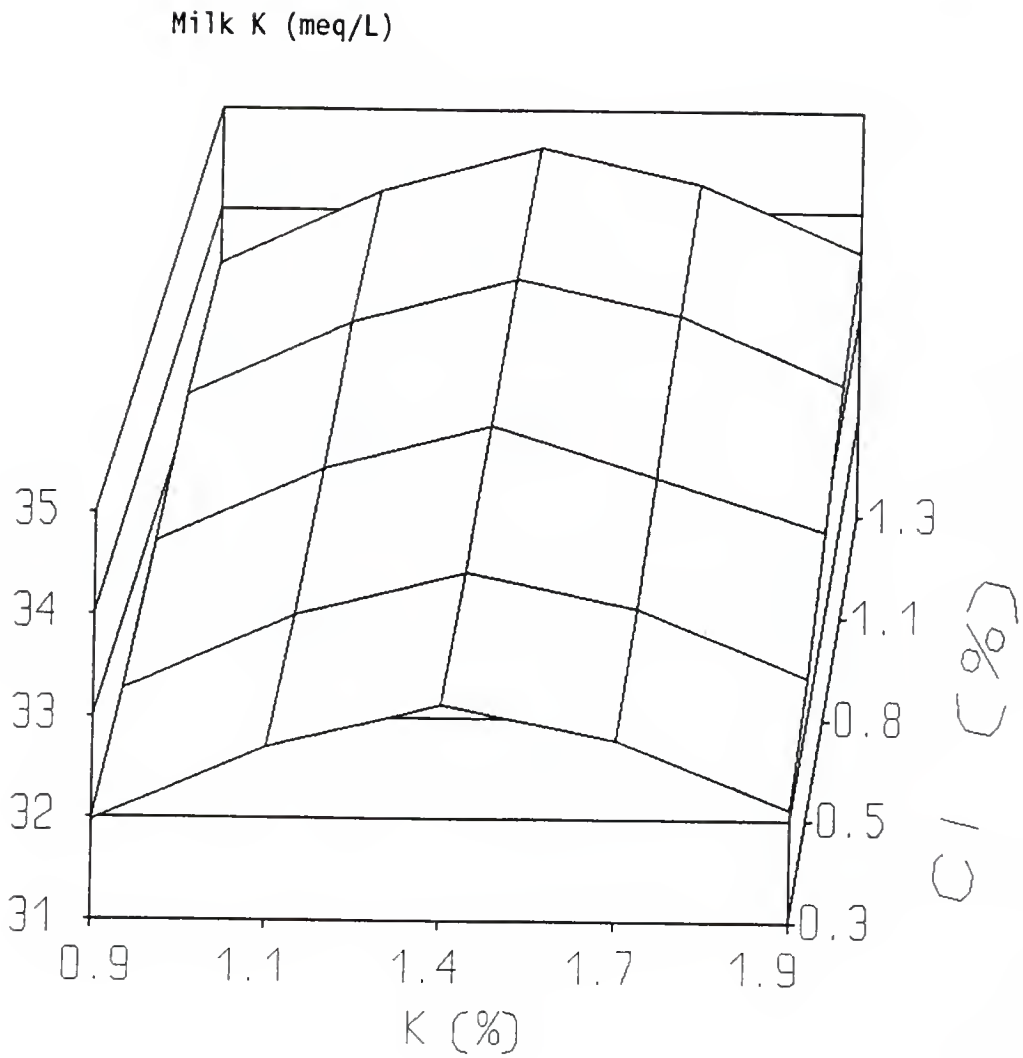

Figure 3-23. Response surface for milk K (MLK) plotted against dietary K and Cl with Na fixed at .55%. Reduced model with SE for each coefficient in parentheses: MLK = 24.16 - .38 (1.00) Na + 12.34 (.22) K + 1.50 (.67) Cl - 4.36 (1.86) K^2. R^2 = .45. Mean and SEM for MLK = 33.44 and .67 meq/L.

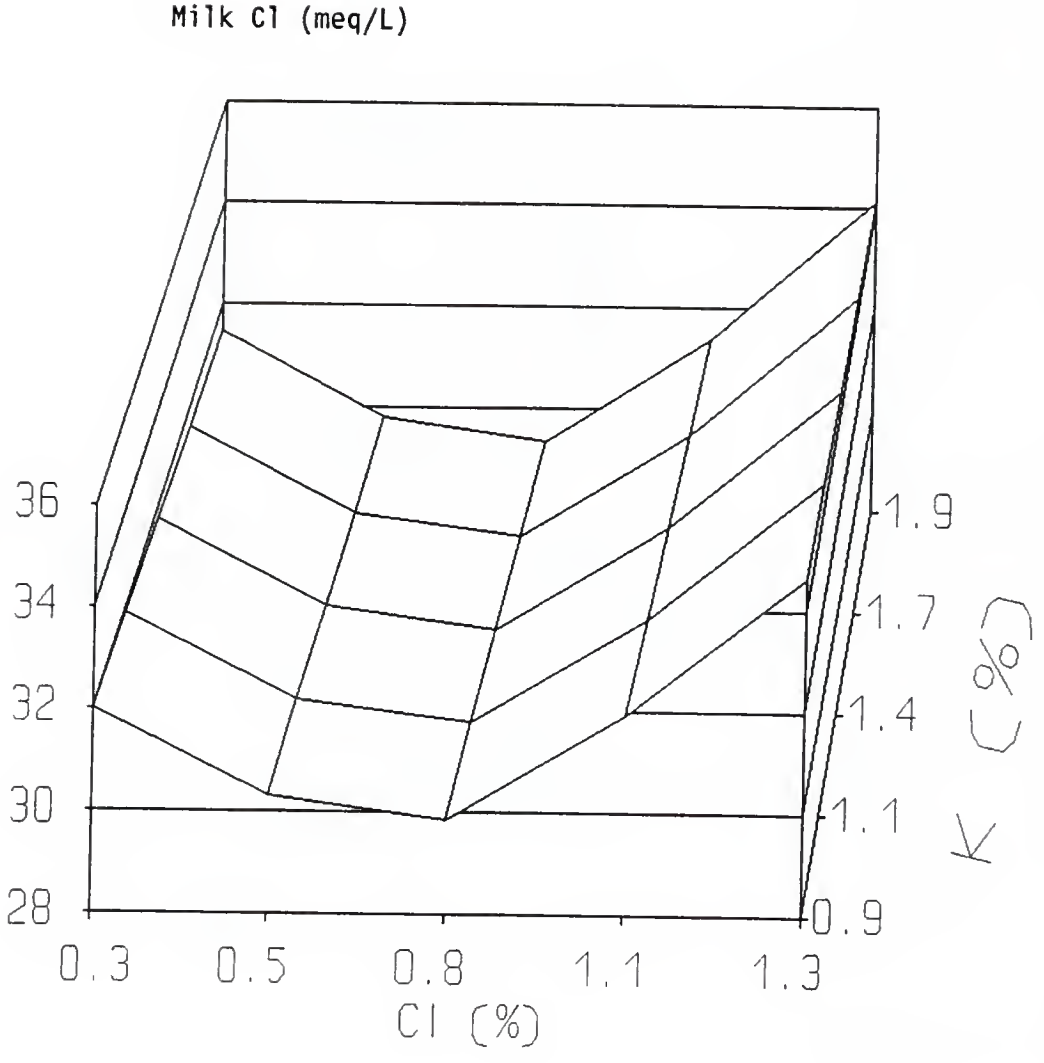

Figure 3-24. Response surface for milk Cl (MLCl) plotted against dietary Cl and K with Na fixed at .55%. Reduced model with SE for each coefficient in parentheses: MLCl = 38.42 - 2.50 (1.82) Na - .52 (1.04) K - 19.46 (8.72) Cl + 13.84 (5.69) Cl^2. R^2 = .50. Mean and SEM for MLCl = 30.53 and 1.23 meq/L.

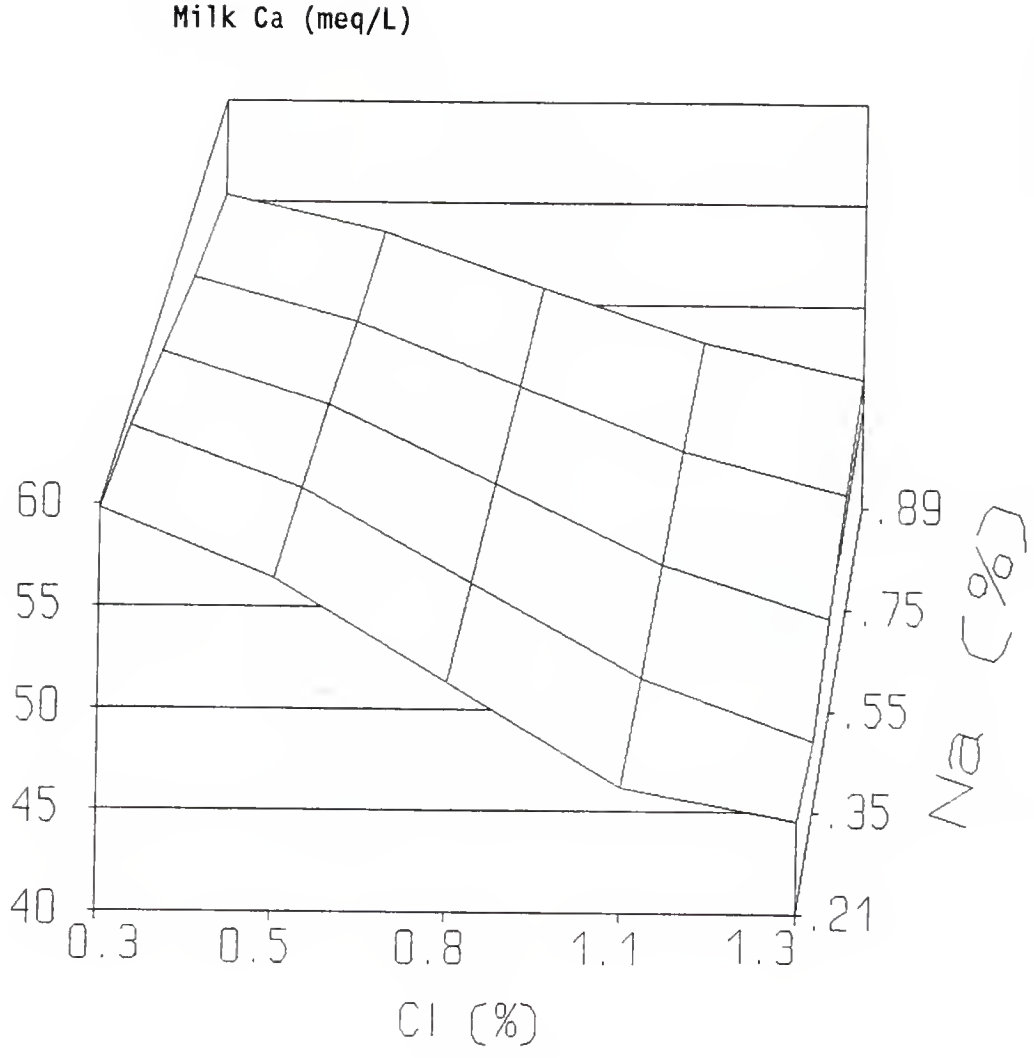

Figure 3-25. Response surface for milk Ca (MLCa) plotted against dietary Cl and Na with K fixed at 1.4%. Reduced model with SE for each coefficient in parentheses: MLCa = 67.76 + 16.42 (26.57) Na - 17.08 (8.51) K - 22.17 (10.73) Cl - 32.05 (19.99) Na^2 + 12.52 (10.59) Na x K + 11.96 (7.55) K x Cl. R^2 = .85. Mean and SEM for MLCa = 49.59 and 2.04 meq/L.

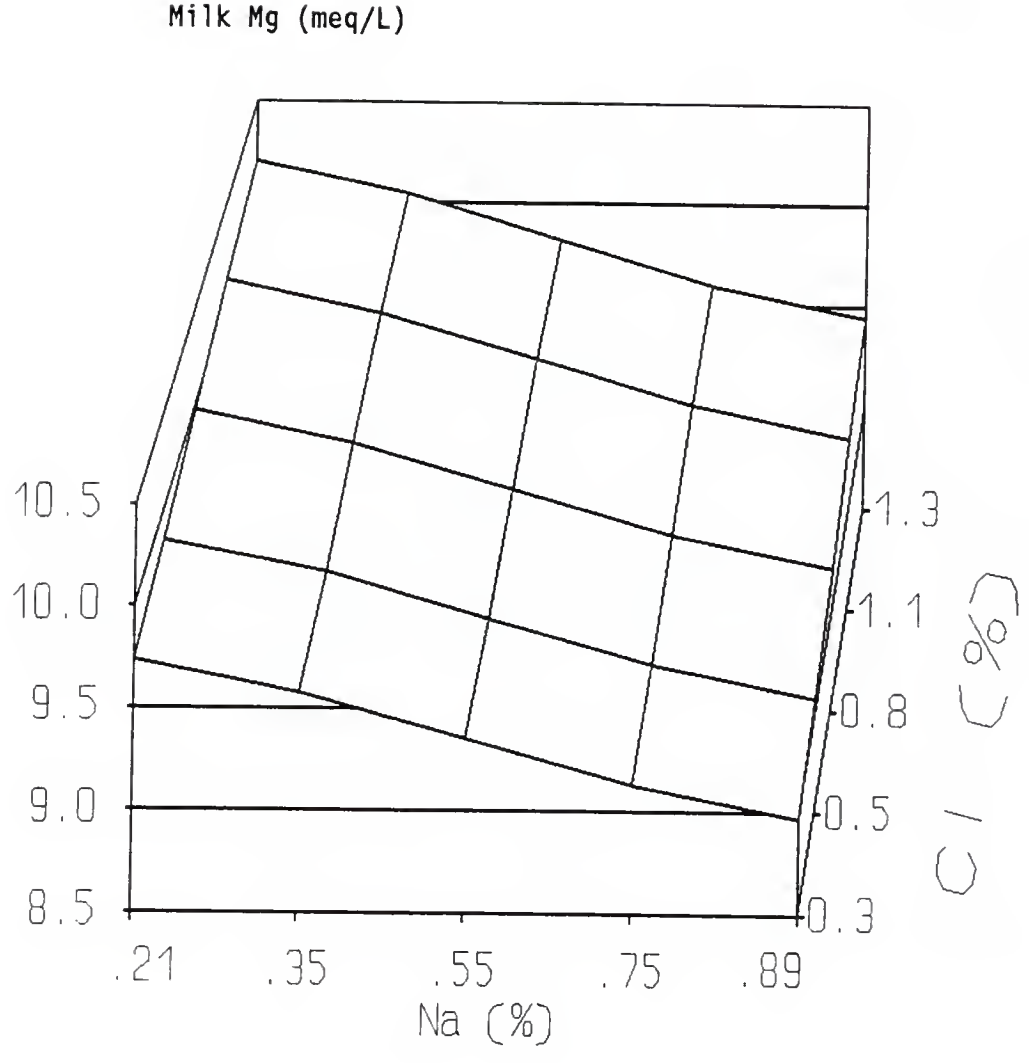

Figure 3-26. Response surface for milk Mg (MLMg) plotted against dietary Na and Cl with K fixed at 1.4%. Reduced model with SE for each coefficient in parentheses: MLMg = 9.28 - 1.12 (.42) Na + .39 (.24) K + .48 (.28) Cl. R^2 = .48. Mean and SEM for MLMg = 9.58 and .28 meq/L.

Determining optimal dietary concentrations of Na, K and Cl was an objective. However, milk fat was the only response variable that was maximized by a single concentration of Na, K and Cl within the range of dietary concentrations used. Most of the responses were influenced by interrelationships among dietary Na, K and Cl. The importance of interrelated effects among Na, K and Cl was a key finding in this study. These will be addressed following a discussion of the linear and quadratic effects of each mineral.

Linear and Quadratic Effects of Dietary Sodium

Regression models indicated that dietary Na in the range of .21 to .89% had a positive linear effect on 3.5% FCM yield independent of dietary concentrations of K and Cl. Mallonee et al. (1982) reported an increase in DMI and MY with increasing dietary Na (from .16 to .70% Na). Because diets were not isochloridic in that study, responses may not have been attributable wholly to Na. In a study with equal concentrations of dietary Cl (Schneider et al., 1986), it was reported that increasing dietary Na above NRC (1978) recommendations (from .18 to .55% using either NaCl or $NaHCO_3$ as the source of Na) improved MY and FCM yield. In agreement with the findings in the present study, they found that low concentrations of Na (.18%) limited MY and FCM yield production.

The positive MF response to increasing dietary Na from .21 to .55% is in accord with earlier work (Rogers et al., 1982; Escobosa et al., 1984; Kilmer et al. 1981; and Schneider et al., 1986). Although diets in this study were not equalized in carbonate content, they were not fat

depressive (MF mean 3.45%) which supports the suggestion by Schneider et al. (1986) that lactational response to dietary Na may be due partly to Na moiety per se and not wholly to buffering effects. Carbonate salts were used to manipulate dietary Na and K because they have less buffering potential (Herod et al., 1978) and contribute more Na and K than bicarbonate salts.

In contrast to Rogers et al. (1982a) a positive linear effect of dietary Na on MP was not observed in this study. Rogers et al. (1982a) reported that cows consuming 2% $NaHCO_3$ produced more total milk protein than cows fed a basal diet without $NaHCO_3$.

In agreement with findings in the present experiment, Nestor et al. (1988) found a similar decline in serum K with increasing dietary Na. In contrast to the current report, Escobosa et al. (1984) and O'connor et al. (1988) reported higher concentrations of K in blood plasma of cows fed increasing dietary amounts of Na (from sodium bicarbonate and sodium chloride, respectively). Erdman et al. (1980) reported that increasing dietary Na from .31 to .52% with .42% K elevated serum K but not with .84% K. The lack of a linear effect of dietary Na on plasma K may have been because diets were not isochloridic in those studies. However, Schneider et al. (1986) equalized Cl concentrations and saw no effect of sodium bicarbonate on plasma K. The reason for the negative linear effect of dietary Na on milk Mg observed in this study is unknown. O'Connor et al. (1988) saw no effect of dietary Na (.24 versus .62%) on milk Mg.

Plasma K, Ca, whole blood K and base excess responded quadratically to dietary Na. Base excess in blood was maximal at .60% dietary Na.

Although blood H^+, HCO_3^- and anion gap were not affected directly by dietary Na concentration ($P > .1$), single blood samples may not have been sufficient to detect differences. Also, maintenance of physiological acid-base status is high on the list of homeostatic priorities (Kronfeld, 1979). Nonetheless perturbations in acid-base status and mineral metabolism were evident. Lower BE and greater plasma Cl, plasma Ca, plasma K, and whole blood K at low (.3 and .35%) vs. middle (.55%) concentration of Na indicated that additional dietary Na was needed to offset subclinical acidosis. Blood base excess, which represents the metabolic component of blood H^+ (Kleinman and Lorenz, 1989), is negatively correlated with metabolic acidosis. Plasma Cl is positively correlated with metabolic acidosis (Lunn and McGuirk, 1990). Flux of K into red blood cells and reattachment of Ca to bone and plasma proteins as H^+ stress lessened could explain the decline in plasma K and Ca. Removal of K and Ca from plasma would be necessary to maintain electrical neutrality because plasma Cl concentrations linearly decreased as dietary Na increased to .55%. Urine samples and more blood samples would be needed to completely understand the physiological response to dietary Na. However, the direct effect of dietary Na on 3.5% FCM [independent of dietary K and/or Cl] coupled with its probable role in acid-base status indicates the need to reevaluate Na recommendations for optimal lactational performance of dairy cattle.

Linear and Quadratic Effects of Dietary Potassium

The only linear or curvilinear effects of K (independent of dietary Na and Cl) were on whole blood Ca, whole blood Cl and milk K. Other

responses apparently were masked by Na x K and K x Cl interactions. These interactions may help resolve discrepancies among published reports on requirements and optimal allowances of dietary K. Reports on the requirements or recommendations of dietary K for lactating dairy cattle have been a subject of controversy. Reports have included .5% (Ward, 1966), .7% (NRC, 1971), .8% (Dennis et al., 1976; Dennis and Hemken, 1978; NRC, 1978; Erdman et al., 1980), .9% (NRC, 1989), 1.0% (Linsner, 1980) and 1.2% Bolenbaugh (1977). Even greater requirements have been reported for cows in heat stress (Mallonee et al., 1985; Schneider et al., 1984b; Schneider et al., 1986; West et al., 1987b).

The K x Cl interaction in this study indicates that responses to dietary K concentration depended upon dietary Cl concentration. It is probable that some of the discrepancies among published K recommendations are due to differing dietary Cl concentrations used. Dietary Cl concentrations were reported infrequently in previous studies which in retrospect would have been very valuable information.

Increased dietary K led to a linear decrease in whole blood Na which likely represented a reduction in red blood cell Na considering that dietary K did not affect plasma Na. Increasing dietary K also elevated whole blood Ca. Because plasma Ca was not affected by dietary K, the rise in whole blood Ca probably was due to increased red blood cell Ca. Increasing dietary K elevated milk K (maximum at 1.4% K) and decreased milk Ca which suggests that the mammary gland is involved in the homeostasis of body K.

Linear and Quadratic Effects of Dietary Chloride

Linear effects of dietary Cl on lactational performance apparently were masked by Na x Cl and K x Cl interactions. Few studies have examined the association between dietary Cl and lactational performance of dairy cattle. Fettman et al. (1984b) concluded that the requirement for Cl was above .1%. In their published figures, average daily MY for cows fed .45% Cl appeared lower but was not significantly different from cows fed .27% Cl. Dietary concentrations of K used by them were lower than in the present report and because of the K x Cl detected here, a difference in optimal dietary Cl in the two studies would not be unexpected. Underwood (1981) suggested that Cl requirement should be substantially higher than the Na requirement because cows milk contains more than twice as much Cl as Na.

Milk fat percentage responded quadratically to increasing dietary Cl. Coppock et al. (1979), Fettman et al. (1984a), and Fettman et al. (1984b) failed to detect significant effects of dietary Cl on MF but these studies used short periods, continuous designs with relatively few cow numbers, and lower dietary Cl concentrations than in the present study. The optimal concentration of dietary Cl for MF (independent of Na and K concentrations) in this study was .69%. The recommended allowance of dietary Cl (NRC, 1989) for milk fat synthesis may need further investigation.

Like dietary Na, Cl influenced acid-base status. Increasing dietary Cl depressed blood HCO_3^-, and thus the cows ability to buffer H^+ ions. An association between dietary Cl and acidosis has been shown in several other studies (Coppock, 1986). In Escobosa et al. (1984) cows fed

diets with 1.65% Cl had lower blood HCO_3^-, pH, pCO_2 and base-excess compared to those fed control diets with .31% Cl.

In the present study, milk K increased linearly and milk Mg decreased linearly with increasing dietary Cl. Plasma Cl and milk Cl responded quadratically to increasing dietary Cl. As Coppock et al. (1982a) suggested, the obligatory demand of the lactating mammary gland for macrominerals may facilitate homeostatic mechanisms to differences in dietary intake.

Two Way Interrelationships

The Na x K interrelationship provides evidence for a sparing effect of the two cations for each other. Increasing dietary Na resulted in a greater increase in DMI when dietary K concentration was low than when high. Dietary Na spared dietary K in other species. In poultry and rats, additional dietary Na spared a portion of the K requirement (Kumpost and Sullivan, 1966; Burns et al., 1953; Grunert et al., 1950). Fontenot et al. (1960) reported that additional dietary Na depressed K absorption in lambs. Increasing dietary K intake in sheep resulted in an increase in fecal Na (Suttle and Field, 1967). Scott (1970) found that high dietary K impaired intestinal absorption of Na and low dietary K increased urinary Na excretion in cattle. Campbell and Roberts (1965) reported that apparent intestinal absorption of Na in heifers was impaired by high dietary concentration of K but lower concentrations of K increased urinary loss of Na. Scott (1967) observed that an increase in the rumen fluid concentration of one of these ions is accompanied by a reciprocal decrease in the other, resulting in an

almost constant meq concentration of the sum of Na plus K. Johnson et al. (1971) observed an interaction between dietary Na and K on microbial populations in the rumen. Most of the published Na x K interactions were related to absorption which could explain why DMI was influenced by a dietary Na x K interaction.

In lactating cattle, studies on the relationships between Na and K have not to this point revealed a sparing effect of dietary Na and K on each other. Erdman et al. (1980) found no benefit of additional Na (0.52 versus .31%) with either low (0.42%) or adequate (0.84%) K. O'Connor et al. (1988) also reported no benefit of additional Na (0.24 versus 0.62%) with either 1.14 or 1.59% K. Chloride was not equalized across diets in those studies which could explain the lack of effects. Martens and Blume (1987) observed in vivo that Na and Cl absorption in sheep was coupled by a dual exchange mechanism of Na^+/H^+ and Cl^-/HCO_3^- and was related to K concentration in the rumen. An alteration in the relative amounts of dietary Na and K thus could be expected to affect acid-base status of the gastrointestinal tract.

One of the main interrelationships discovered in this study was a dietary cation by anion interaction demonstrating the need to balance or couple dietary cations with anions. In general, responses to increasing dietary cation (Na or K) was most beneficial when coupled with increasing dietary anion (Cl). If dietary Na and/or K was increased without a concomitant increase in Cl, and vice versa, response was negative. This was evident for several variables related to lactational performance (DMI, FCM, MP, and BWG).

Predicted DMI was highest when both Na and Cl concentrations were raised in unison (Na x Cl interaction). If dietary Na concentration was low and Cl was high (or vice versa) predicted DMI was much less than when both were high or both were low. Coppock et al. (1982a) proposed that a dietary ratio of Na:Cl was important, perhaps analogous to the Ca:P ratio. However, no differences in milk yield or milk composition from cows fed ratios between 1:1 and 4:1 were observed. Hurwitz et al. (1973) reported maximal growth of chicks fed a 1:1 (percentage basis) ratio of dietary Na and Cl.

The pCO_2 response surface plotted against dietary Na and Cl was similarly shaped to the same plot for DMI. Partial pressure of CO_2 declined with increasing dietary Na at low dietary Cl concentration but increased at high dietary Na concentration. According to the Henderson-Hasselbach equation (Kleinman and Lorenz, 1989), blood HCO_3^- and pCO_2 ratio must remain constant for blood pH to remain constant. In an attempt to maintain constant ratio of blood HCO_3^- and pCO_2 and thus constant blood pH, pCO_2 would need to decline when HCO_3^- declines. Respiration would need to increase to expel CO_2 from the lungs in order to reduce pCO_2. Because homeostatic mechanisms to control respiration and acid-base status take priority over lactational performance (Kronfeld, 1979) it is likely that a reduction in intake occurred due to alterations in acid-base status and an imbalance in dietary Na and Cl and. The reasoning for this is as follows. Chloride and HCO_3^- excretion are reciprocally related in the kidneys (Kleinman and Lorenz, 1989). Excretion of one ion is coupled to the reabsorption of the other. With high concentration of Na and low concentration of Cl at the

kidney tubule, HCO_3^- ions would replace the Cl normally excreted with Na in the urine. When dietary Cl concentration is increased to match the high dietary Na, Cl then would be available to accompany Na in the urine, HCO_3^- would be conserved, acid-base status would be unaltered and intake would proceed normally.

The dietary Na x Cl interaction for plasma Na was opposite that of DMI and blood pCO2 and revealed that with increasing dietary Na, plasma Na increased when dietary Cl concentration was low, but decreased when Cl was high. Plasma Na has been shown to decline postfeeding (Tucker et al., 1988b) and because blood samples were taken several hours after feeding (assuming cows did not eat much during the night), plasma Na response in this study could be a function of feeding. Reports on the effect of dietary Na on plasma Na have been variable.

A dietary Na x Cl interaction did not influence 3.5% FCM yield. But a synergistic or coupling effect of cation and anion on 3.5% FCM yield was still present. Potassium instead of Na was the cation that interacted with Cl on 3.5% FCM yield. To date, interrelationships between K and Cl on milk production have not been reported for lactating dairy cattle. Data of Tucker et al. (1988a) provide evidence for a K x Cl interaction on MY. With a relatively high Cl diet (1.25%), there was a positive linear increase in MY to increasing dietary K (from .73 to 1.91% K). Lower concentrations of Cl were not tested in combination with that range of dietary K so this cannot be confirmed conclusively from their data.

A dietary K x Cl interaction on growth of weanling pigs was reported by Golz and Crenshaw (1990). The nature of the K x Cl interaction in

that report and the current one was similar. At low concentration of K an increase in dietary Cl depressed pig growth, whereas at high concentration of K a similar increase in Cl improved growth. A possible explanation for the dietary K x Cl interaction in this study may be derived from the findings of Paquay et al. (1969b) who observed a positive correlation between K and Cl in the urine of cows. They suggested that because ruminants are herbivores they consume greater than required K from forage. The bovine kidney is adapted to eliminate excess K, but to maintain electrical neutrality of the urine, additional Cl is needed to accompany excess K cations. If dietary Cl is insufficient relative to dietary K, animal performance potentially could be altered.

Analogous with the influence that K x Cl had on 3.5% FCM, MP declined with increasing dietary K at low Cl concentration but increased with increasing dietary K at high Cl. Milk protein was reduced by more than 10% at the extremes of the response surface (Figure 3-8). Schneider et al. (1986) reported a decline in milk protein when dietary K increased from 1.3% to 1.8% but in contrast to findings in the current study, this decline occurred at relatively high Cl (calculated to be 1.25%). Source of dietary K in the high K diet of that study was KCl (vs. K_2CO_3 in this study) which may explain the difference in response. Tucker et al. (1988a) observed a decrease in MP with increasing dietary cation Na and/or K when Cl was simultaneously reduced. West et al. (1987b) also reported a decline in milk protein with increasing dietary K from .93 to 1.53%. Escobosa et al. (1984) reported that cows fed diets with 2.28% $CaCl_2$ had lower MP than those fed diets with 1.7%

$NaHCO_3$. Pradhan and Hemken (1968) noted a decrease in milk protein when dietary K concentration increased from deficient levels to 1.8%. The effects of dietary K and Cl on milk protein are not understood. Further investigations are warranted considering the current and future emphasis of pricing milk on protein content.

Body weight gain also was influenced by dietary K x Cl interaction. Body weight gain decreased with increasing dietary K at low concentration of Cl but increased with increasing dietary K at high concentration of Cl. Dennis et al. (1976) and Dennis and Hemken (1978) reported differences in BWG due to increasing dietary K from .69 to .97% and from .55 to .66% K, respectively. Both studies used KCl as the source of K which supports the beneficial effect of coupling dietary cation (K) with anion (Cl) that we observed in the present study. Escobosa et al. (1984) reported that cows fed diets with 2.28% $CaCl_2$ had lower DMI than those fed a diet with 1.7% $NaHCO_3$.

Dietary K x Cl interaction also influenced mineral metabolism (plasma Na and whole blood Mg). As dietary K increased, plasma Na and whole blood Mg decreased when dietary Cl concentration was low, but increased when dietary Cl concentration was high.

Three Way Interrelationships

To account for potential interrelationships among dietary Na, K and Cl, researchers have attempted to relate acid-base status and animal performance to a linear combination of these three minerals. The basic theory behind this concept is that all body fluids must remain electrically neutral and because absorbed, non metabolizable ions (such

as Na, K and Cl) contribute positive and negative charges to the system, they affect electrical balance of the body. This affects acid-base status which can in turn influence animal performance (i.e., growth or lactation). From work with poultry and swine, it was determined that Na, K and Cl were the most important elements of this expression (Mongin, 1980).

This concept was first evaluated in lactating dairy cattle by Tucker et al. (1988a). They fed diets with -10, 0, +10 and +20 CAD. The diet with +20 CAD improved DMI by 11% and MY by 9% compared with -10 CAD, independent of the individual minerals (Na, K or Cl) used to vary dietary CAD. Blood, rumen, and urine measures indicated an improvement in acid-base status with high cation (+20 meq) diet as compared with low cation (-10 meq) diet. It was hypothesized that regulating CAD may become a useful tool for improving performance of lactating dairy cattle.

In addition to exploring optimal responses to varying concentrations of dietary Na, K and Cl, and their interrelationships, a final objective of this study was to examine how CAD related to response differences. Calculated CAD values are in Table 3-3 and ranged from +12 to +62. This range is typical of values found in practical commercial diets. For reference, if CAD was calculated using average NRC (1989) recommendations for Na, K and Cl, +25 CAD would be the recommendation for lactating dairy cattle.

To determine the influence of CAD, general linear models procedures of SAS (1985) were used to generate models containing discrete effects of cow and period and treatment effects partitioned into continuous

effects of CAD. Mathematical models were fit with linear, quadratic, and cubic CAD terms and then reduced by sequentially removing terms (from highest to lowest order) that did not contribute to the significance of the regression ($P > .1$). Dry matter intake ($P = .05$; Figure 3-27), BWG ($P = .06$; Figure 3-30), and blood pCO_2 ($P = .06$; Figure 3-33) responded in a cubic fashion to CAD. Yield of 3.5% FCM ($P < .05$; Figure 3-28), MP ($P = .09$; Figure 3-29), and blood HCO_3^- ($P=.09$; Figure 3-31) responded in a quadratic fashion to CAD. Blood base excess ($P = .08$; Figure 3-32), plasma Cl ($P < .05$; Figure 3-34), and milk Cl ($P = .07$; Figures 3-35) responded in a linear fashion to CAD.

West et al. (1990) compared lactation diets with +2.5, +15, +27.5 and +40 CAD and observed a similar cubic DMI response to increasing CAD. From +2.5 to +40 CAD, West et al. (1990) reported a linear increase in MY and a cubic increase in BE. In another study by this group (West et al., 1991), DMI and blood pH increased linearly and blood HCO_3^- increased cubically with increasing CAD (from +10 to +45.1 CAD). As in Tucker et al. (1988a), there was a negative linear relationship between CAD and plasma Cl in the present study. In Tucker et al. (1988a) and West et al. (1990 and 1991) production and acid-base status responses were influenced negatively by low (between -10 and +10) CAD.

In the present study, regression models predicted that maximum responses were always between +38 and +48 CAD. Derivatives indicated that 3.5% FCM was maximized with +41 CAD, milk protein with +38 CAD, and blood HCO_3^- with +47.6 CAD. In support of results of Tucker et al. (1988a) and West et al. (1990 and 1991) results of the present study also demonstrated negative influence of low CAD on acid-

DMI (kg/d)

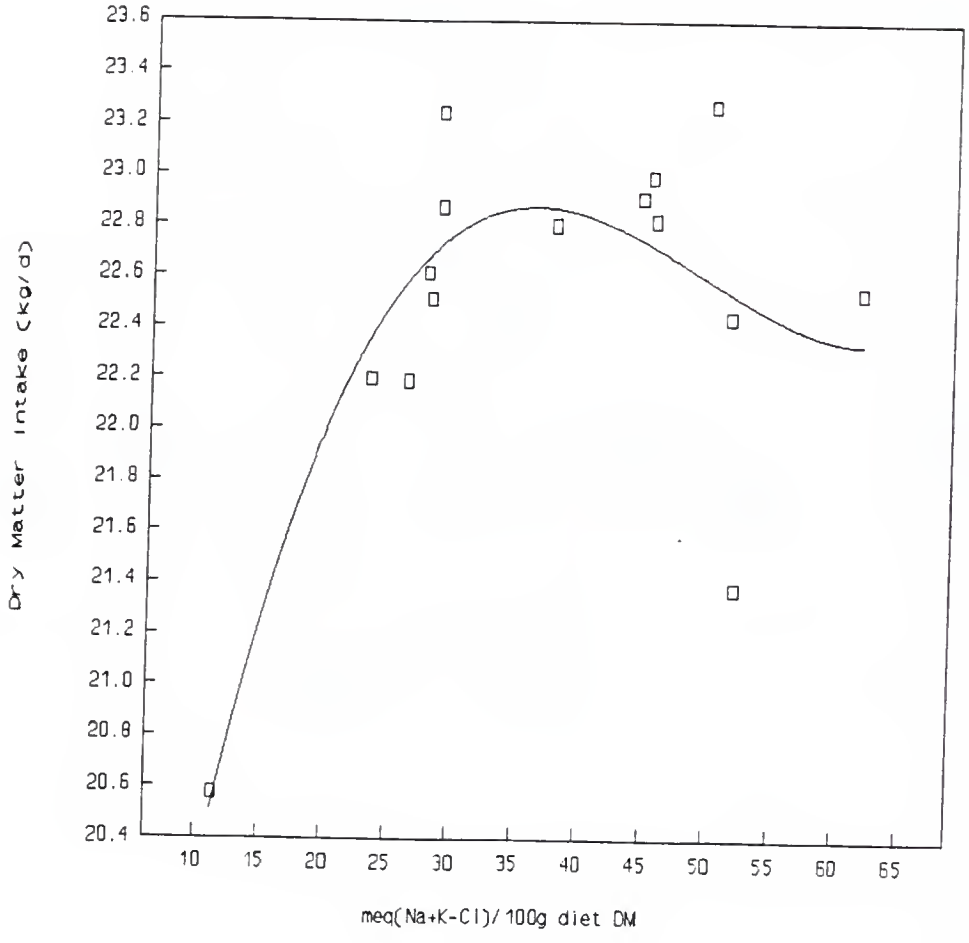

Figure 3-27. Dry matter intake (DMI) response to CAD. Reduced model with SE for each coefficient in parentheses: $DMI = 16.93 + .4078$ (.12) CAD - .000892 (.003) CAD^2 + .0000605 (.00003) CAD^3. $R^2 = .65$. Mean and SEM = 22.5 and .38 kg/d.

Yield of 3.5% FCM (kg/d)

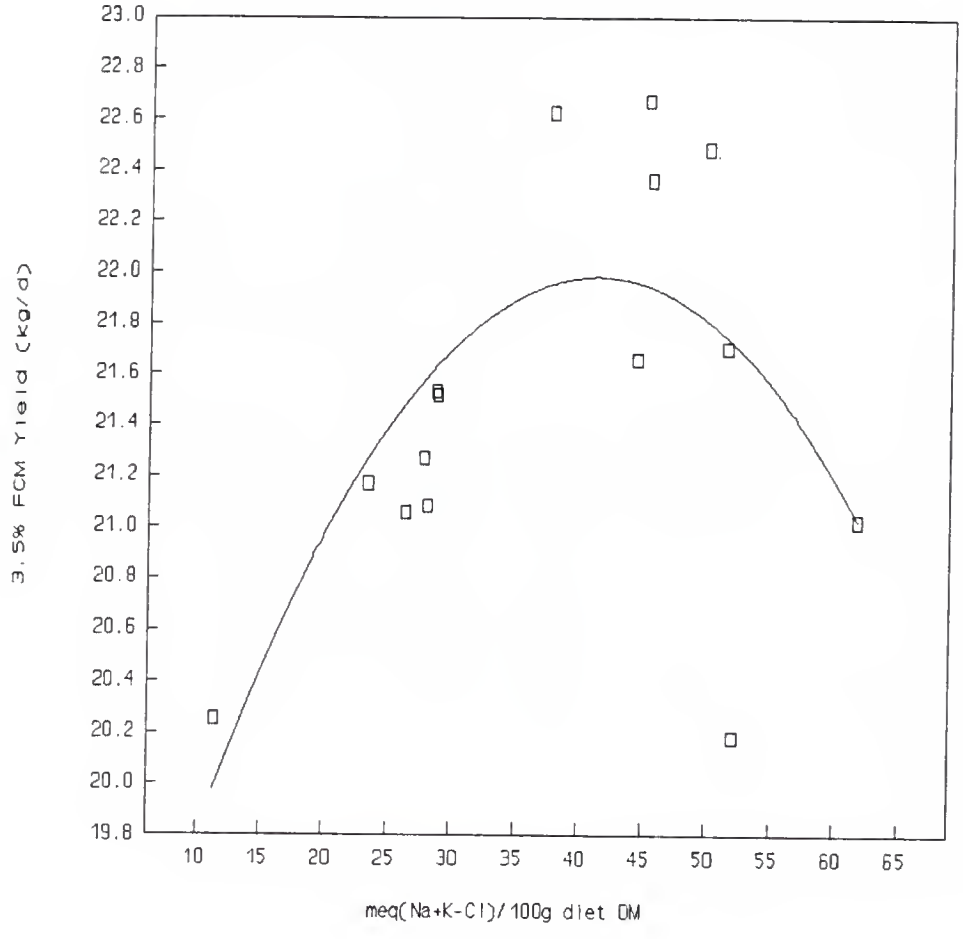

Figure 3-28. Yield of 3.5% FCM response to CAD. Reduced model with SE for each coefficient in parentheses: 3.5% FCM yield = 18.15 + .1861218 (.07) CAD - .00225853 (.0009) CAD^2. R^2 = .45. Mean and SEM = 21.5 and .67 kg/d.

Milk Protein (%)

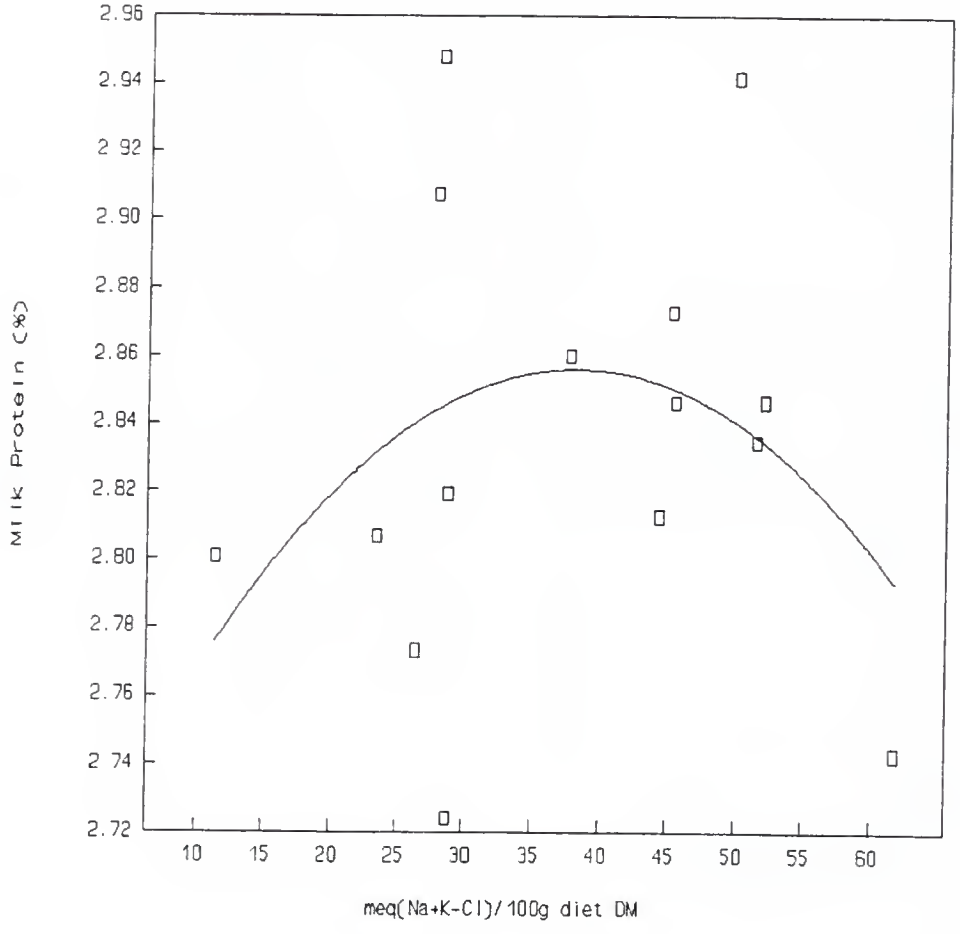

Figure 3-29. Milk protein percentage (MP) response to CAD. Reduced model with SE for each coefficient in parentheses: MP = 2.69 + .0085859 (.005) CAD - .00011255 (.00007) CAD^2. R^2 = .14. Mean and SEM = 2.83 and .05%.

Body Weight Gain (kg/d)

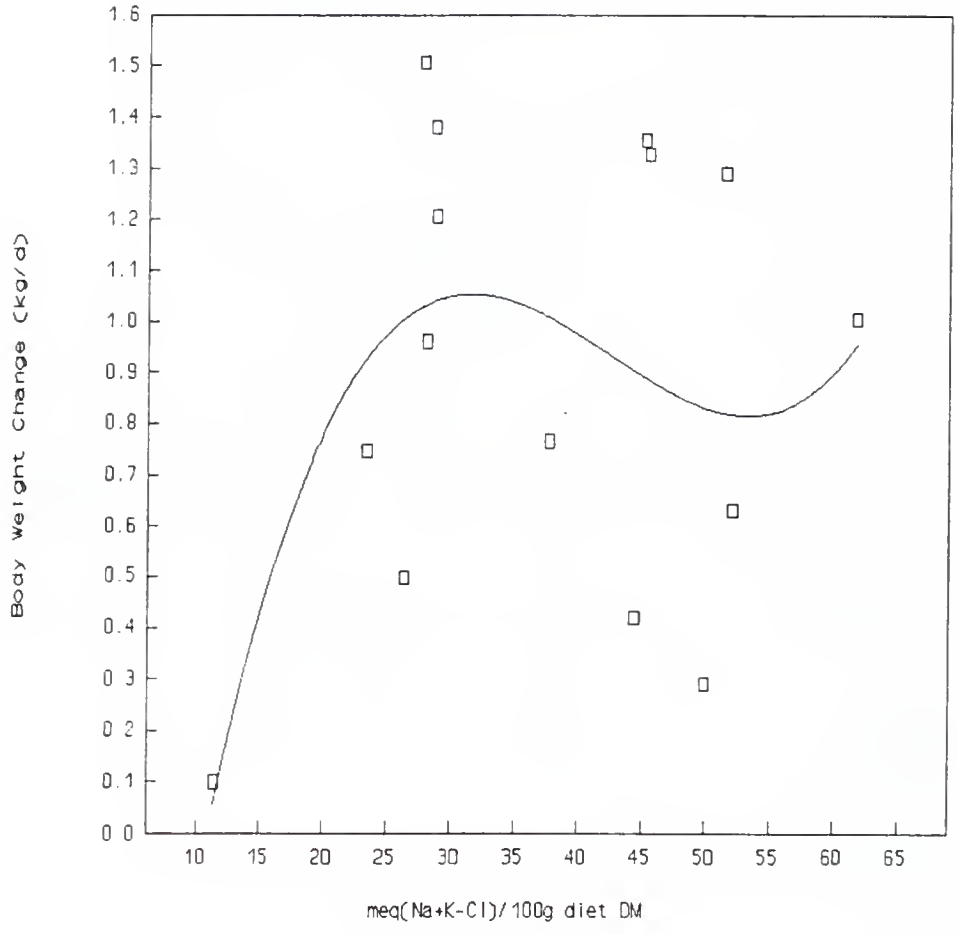

Figure 3-30. Body weight gain (BWG) response to CAD. Reduced model with SE for each coefficient in parentheses: BWG = -1.9 + .23489 (.09) CAD - .005929 (.003) CAD^2 + .00004662 (.00002) CAD^3. R^2 = .31. Mean and SEM = .90 and .30 kg/d.

Blood Bicarbonate (meq/L)

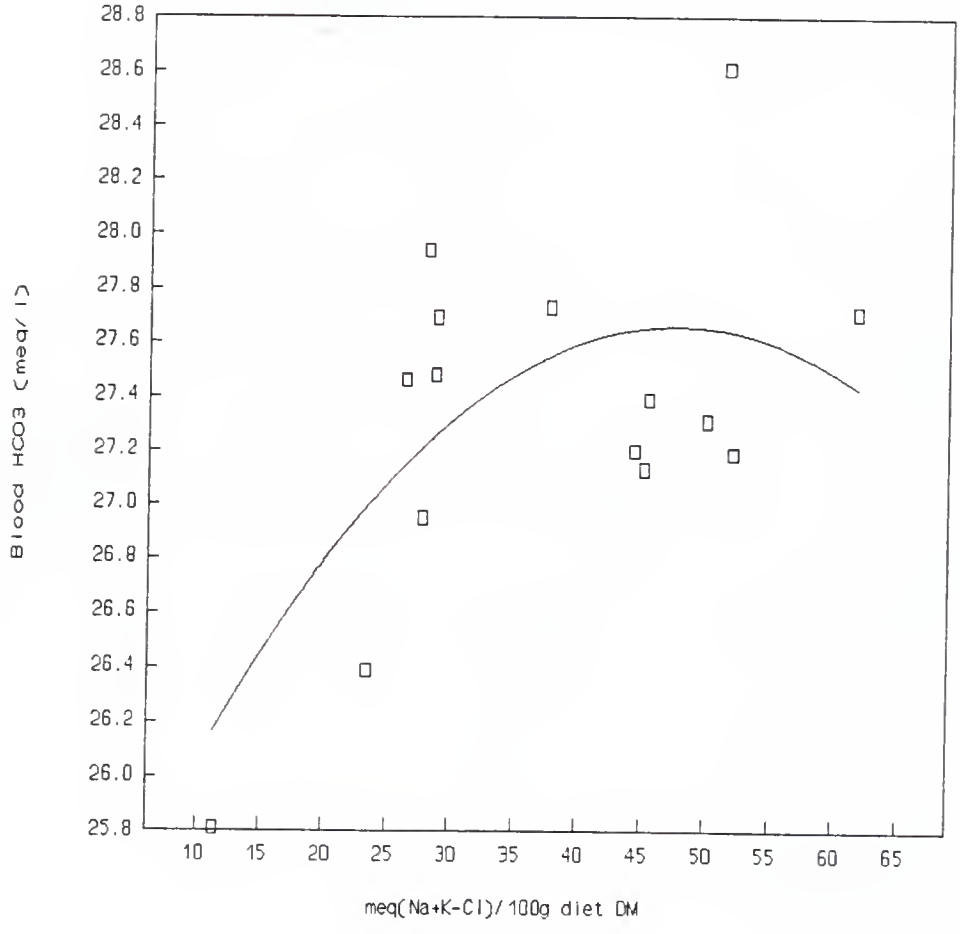

Figure 3-31. Blood bicarbonate (HCO_3^-) response to CAD. Reduced model with SE for each coefficient in parentheses: HCO_3^- = 25.08 + .10844375 (.05) CAD - .00113928 (.0007) CAD^2. R^2 = .41. Mean and SEM 27.33 and .51 meq/L.

Blood Base Excess (meq/L)

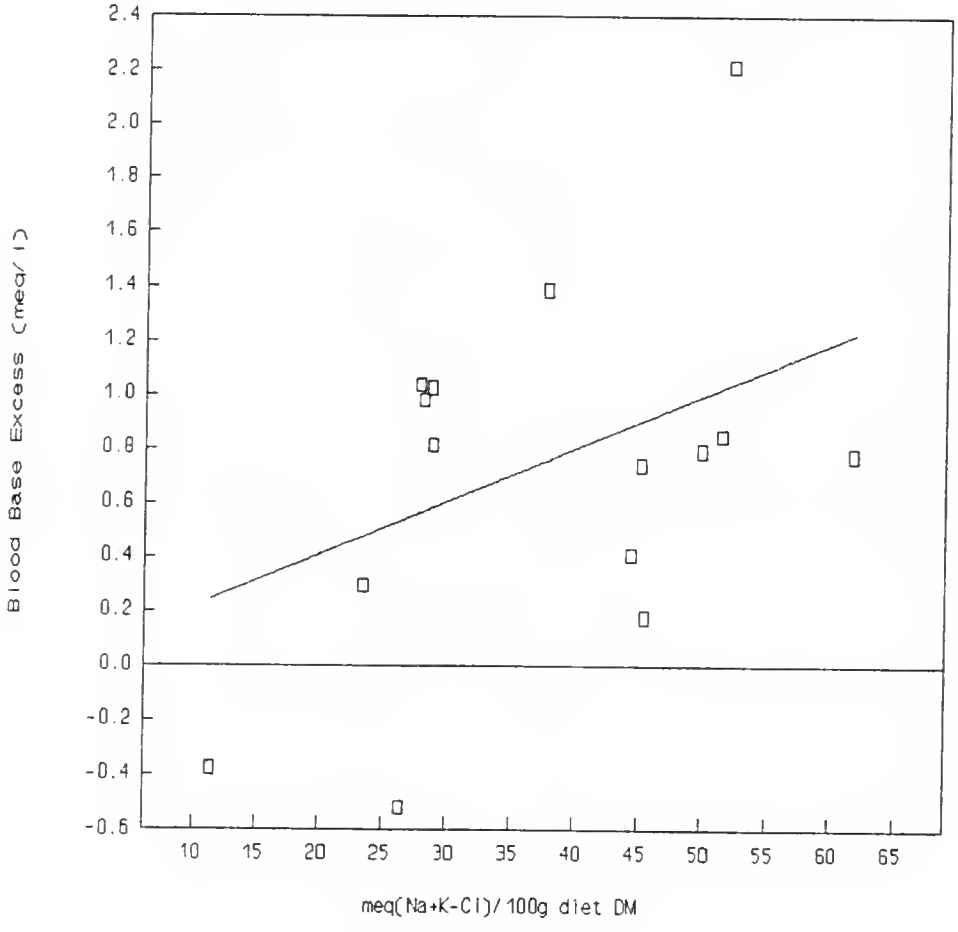

Figure 3-32. Blood base excess (BE) response to CAD. Reduced model with SE for each coefficient in parentheses: BE = .02 + .019478 (.01) CAD. R^2 = .21. Mean and SEM = .71 and .57 meq/L.

Blood pCO_2 (mm Hg)

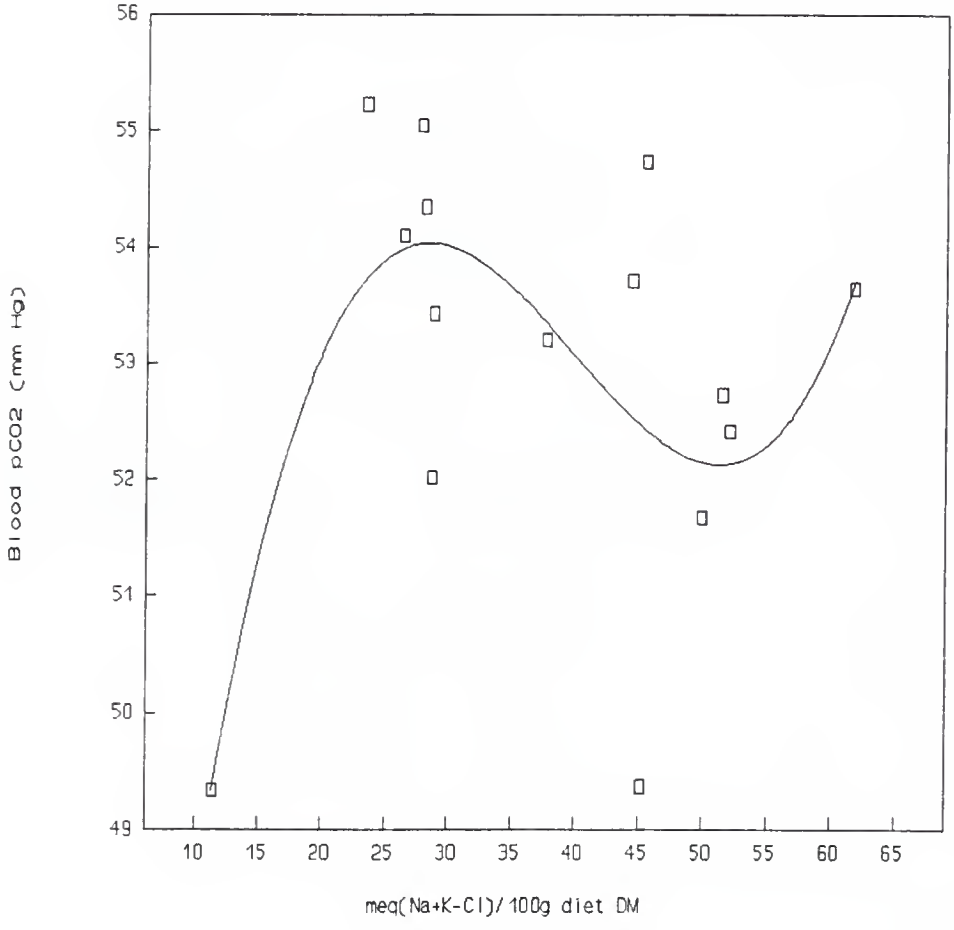

Figure 3-33. Blood pCO_2 (pCO_2) response to CAD. Reduced model with SE for each coefficient in parentheses: pCO_2 = 37.98 + 1.387579 (.63) CAD -.0379905 (.02) CAD^2 + .00031805 (.0002) CAD^3. R^2 = .46. Mean and SEM = 53.0 and 2.14 mm Hg.

Plasma Cl (meq/L)

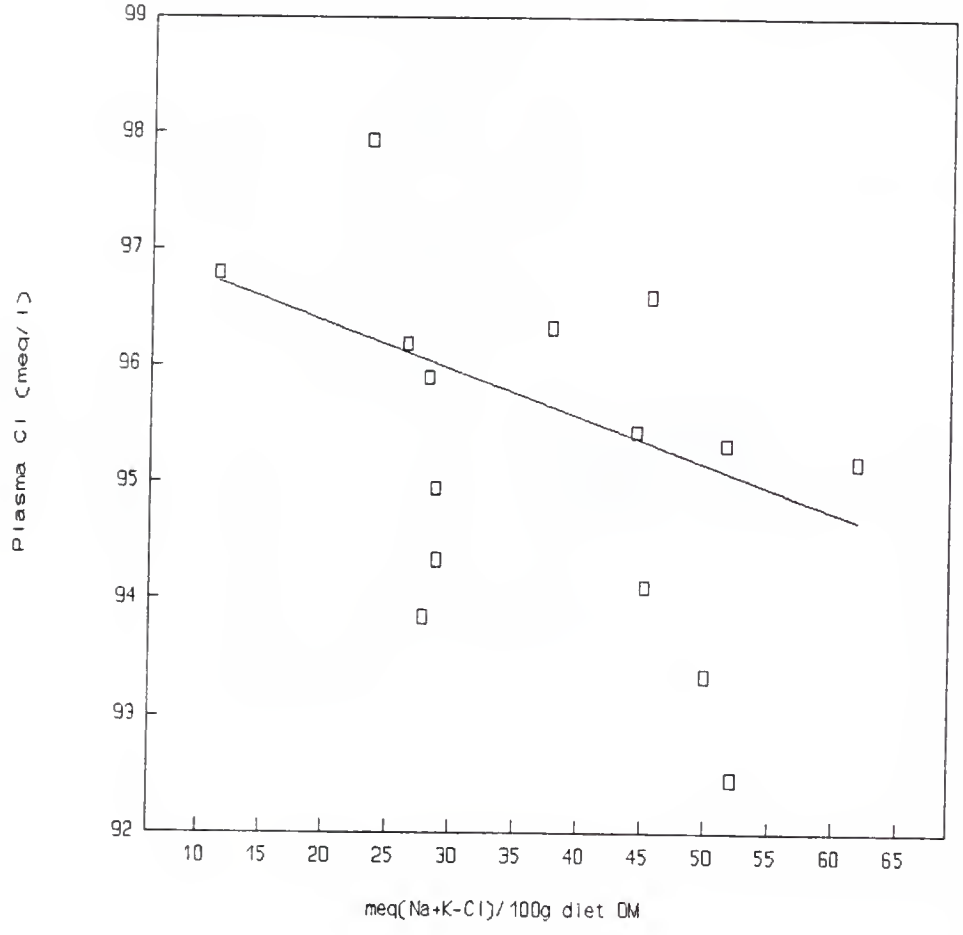

Figure 3-34. Plasma Cl (PCl) response to CAD. Reduced model with SE for each coefficient in parentheses: PCl = 97.19 - .047947 (.02) CAD. R^2 = .23. Mean and SEM = 95.25 and 1.07 meq/L.

Milk Cl (meq/L)

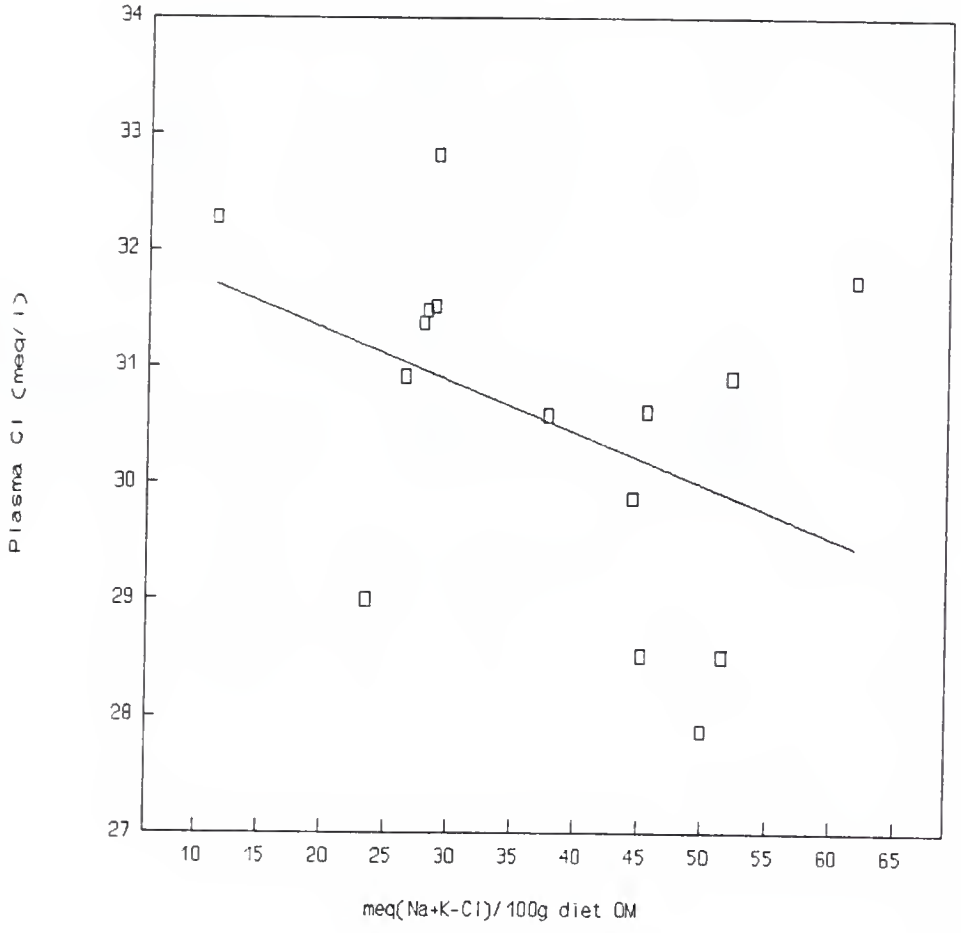

Figure 3-35. Milk Cl (MLCl) response to CAD. Reduced model with SE for each coefficient in parentheses: MLCl = 32.22 - .045 (.02) CAD. R^2 = .18. Mean and SEM = 30.53 and 1.23 meq/L.

base status. Results of Tucker et al. (1988a), West et al. (1990 and 1991), and those presented here, indicate that at values below +20 CAD, the CAD expression is a useful measure of acid-base status and lactational performance.

Above +20 CAD additional factors may be responsible for responses to dietary Na, K and Cl. Golz and Crenshaw (1990) who also used response surface methodology to explore interrelationships among dietary Na, K and Cl for growing swine, suggested a plausible reason. The simple CAD expression implies that all ions contribute equally to animal performance. As we have discovered in this study, these minerals do not act independently. This CAD expression correctly separates the effects of cations and anions but it assigns equal value to each ion. If dietary Na and K contributed equally both should have interacted identically with dietary Cl. In this study they did not. It should be noted that with most CAD studies (this one included) dietary carbonate and bicarbonate concentrations are confounded with CAD. Because these salts are used to elevate CAD, CAD effects cannot be separated from well known ruminal and systemic buffering effects (Erdman, 1988; Staples and Lough, 1989) of carbonate and bicarbonate salts.

Conclusions

In this investigation three important findings were made. Firstly, there were linear and curvilinear responses to dietary Na and Cl. Dietary Na had a positive linear effect on MY (independent of dietary K and Cl concentrations) and milk fat percentage responded quadratically

to dietary Cl; MF was maximized with .69% Cl (independent of dietary Na and K). Secondly, interrelationships were abundant, particularly those associated with the two cations (Na and K) and with cation and anion (Na x Cl and K x Cl). The data suggested that dietary Na and K spared one another and that coupling the concentrations of cations and anions was beneficial. Increasing dietary Na or K was most beneficial when accompanied by increasing dietary Cl. These interrelationships were related to blood acid-base status and mineral metabolism. Thirdly, a dietary CAD of +12 resulted in subclinical metabolic acidosis, reduced feed intake and milk production. When CAD was +41, 3.5% FCM yield was maximized.

CHAPTER 4
DIETARY MIXTURES OF SODIUM BICARBONATE, SODIUM CHLORIDE AND POTASSIUM CHLORIDE: EFFECTS ON ACID-BASE STATUS, MINERAL METABOLISM AND LACTATIONAL PERFORMANCE OF DAIRY CATTLE

Introduction

Buffers are used in dairy cattle diets to combat milk-fat depression. It is estimated that more than 50% of all dairy farms in the United States may be using dietary buffers such as $NaHCO_3$ for this purpose (Downer and Cummings, 1987). Animal nutritionist have concluded that dietary buffers reduce ruminal acidity and improve systemic acid-base status (Erdman et al., 1988). In addition to maintaining ruminal pH, non-buffering effects from feeding dietary buffers have been noted (Rogers et al., 1982). These non-buffering effects are from the solute action of buffers. Upon dissociation in the rumen, buffers increase ruminal osmotic pressure and liquid dilution rate (Harrison et al., 1976; Rogers et al., 1979; Rogers et al., 1982a; Rogers et al., 1982b). This, in turn, increases influx of water, accelerates flow of liquid digesta from the rumen (Rogers et al., 1979) and is associated with increased efficiency of fiber digestion (Rogers et al., 1982), microbial protein synthesis (Harrison et al., 1975), and increased organic matter utilization (Croom et al., 1982) in cattle fed high concentrate diets. Schneider et al. (1986) hypothesized that responses to feeding dietary buffers to lactating cows are due both to the HCO_3^- and Na moiety.

There is considerable information on acid-base physiology, mineral metabolism and lactational response to dietary buffers such as $NaHCO_3$

(Erdman, 1988; Staples and Lough, 1989), but very little information exists on other dietary solute sources such as NaCl and KCl. Equally lacking is research information on combinations of dietary mixtures of $NaHCO_3$, NaCl, and KCl. Mixtures of mineral supplements could be developed that contain both the buffering effects of $NaHCO_3$ and solute effects of less expensive mineral salts (such as NaCl and KCl). There may be a mixture of $NaHCO_3$, NaCl, and KCl that equals the buffering and solute potential of $NaHCO_3$ alone. Objectives of the present study were to determine the effects of dietary mixtures of $NaHCO_3$, NaCl and KCl on acid-base status, mineral metabolism and lactational performance of dairy cattle. Also, because the dietary cation-anion difference (CAD) calculated as meq (Na + K - Cl)/100 g diet DM, is related to production and physiological responses of lactating dairy cattle (Tucker et al., 1988a; West et al., 1990; West et al., 1991) and because the differing concentrations of $NaHCO_3$, NaCl and KCl altered CAD, an additional objective of this study was to further explore response relationships to varying CAD.

Materials and Methods

Management

Thirty-six midlactation Holstein cows averaging 123 DIM and 29.75 kg milk/d were fed a 40:13:47 corn silage:whole cottonseed:concentrate total mixed diet (Table 4-1) twice daily at 0800 and 1400 h. The concentrate was mixed monthly in large batches, stored in upright bins and used daily as needed. An electronic door feeding system (American Calan, Inc., NorthWood, NH) was used to record daily feed intake of

TABLE 4-1. Ingredient composition and nutrient analysis of basal (control) diet.

Ingredient	% of DM
Corn silage	40
Whole cottonseed	13
Ground yellow corn	26
Soybean meal	10
Corn distillers dried grains	7
Vitamin-mineral premix[1]	1
Hydrolyzed feather meal	1
SiO_2 (washed sand)	1
Limestone	.45
Trace mineral salt[2]	.25
NaCl	.15
Magnesium oxide	.15
Nutrient Analysis	
NE_L, Mcal/kg[3]	1.76
CP	17.13
UIP[4]	6.88
ADF	21.66
Ca	.83
P	.49
Mg	.36

[1]Contained Ca 32%, P 7%, Mn .25%, Cu .12%, Se .00115%, Vitamin A 136364 IU/kg, Vitamin D_3 5909 IU/kg and Vitamin E 2273 IU/kg.

[2]Contained NaCl 92%, Mn .25%, Fe .2%, Cu .033%, Zn .3%, I .007%, Co .0025%.

[3]Value calculated from chemical analysis.

[4]Value calculated from composition of individual ingredients (NRC,1989).

individual cows. Total mixed rations were made by combining corn silage, whole cottonseed and concentrate immediately before each feeding. A mobile mixing and feeding unit with electronic scales was used to deliver the feed (American Calan, Inc., Northwood, NH). Cows were fed so that 5 to 10% feed (as-fed basis) remained at 0630 h each d. Remaining feed was weighed and removed prior to morning feeding. Cows were housed in an open sided free stall barn equipped with a sprinkler and fan evaporative cooling system. All animals were provided continuous and equal access to exercise lots and drinking water. Milking was at 0500 and 1600 h.

Treatments

All cows received a different treatment in each of three consecutive 28-d periods. Treatments consisted of sodium bicarbonate, ($NaHCO_3$), sodium chloride (NaCl) and potassium chloride (KCl) and mixtures of each (Table 4-2). The one, two and three component mixtures are referred to as primary, binary and tertiary mixtures respectively (Cornell, 1990). Three 100:0:0 (primary) mixtures, three 50:50:0 (binary) mixtures and one 33:33:33 (tertiary) mixture of $NaHCO_3$, NaCl and KCl were formulated to replace one percent of the diet DM. Mixtures were added to a diet already adequate in Na, K and Cl (Table 4-2).

These seven treatments were defined according to a mixtures simplex-centroid design (Cornell, 1990) using a partially balanced incomplete block arrangement (Montgomery, 1984). A feature of the mixtures design is that quantities of each component represent proportionate amounts of a mixture. The eighth treatment (control) was

TABLE 4-2. Composition and nutrient analysis of experimental diets (% of diet DM).

	Treatment[1]							
Ingredient	1	2	3	4	5	6	7	8[2]
$NaHCO_3$	1.0	...	...	.5	.5	...	.33	0
NaCl	...	1.0	...	.5	...	.5	.33	0
KCl	...	...	1.0	...	.5	.5	.33	0
Total	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Na, K and Cl Concentrations								
Na	.64	.85	.36	.76	.44	.54	.52	.32
K	1.10	1.09	1.57	1.10	1.43	1.35	1.25	1.13
Cl	.56	1.34	1.08	.99	.91	1.15	.83	.53
CAD[3]	40	27	25	33	30	26	31	28

[1]Treatments consisted of additions (1% diet DM) of $NaHCO_3$, NaCl and KCl mixtures to basal diet (in place of SiO_2).

[2]Treatment 8 was the control diet; see Table 4-1 for composition.

[3]CAD = meq (Na + K - Cl)/100 g diet DM.

equivalent to treatment diets in all nutrients except Na, K and Cl (Table 4-2). This control diet contained none of the mineral-salt mixtures but still met or exceeded NRC recommendations (1989) for Na, K and Cl. To equalize amount of ash in treatment and control diets, 1% SiO_2 (washed sand) was added to the control diet.

Sample Collection and Analysis

Dry matter intake (DMI), milk yield (MY), and 3.5% fat-corrected milk (3.5% FCM) yield data were collected during the last 2 wk of each period. Two milk samples from each cow were collected at each milking on the last 3 d of each period. One sample, preserved with $K_2Cr_2O_7$, was analyzed for fat and protein percentage (DHIA Testing Laboratory, Raleigh, NC). The other milk sample was frozen (-10 °C) for later mineral analyses. Body weights were recorded after the a.m. milking three mornings immediately prior to the start of the experiment and during the last 3 d of each experimental period.

Corn silage was sampled three times weekly for DM. Adjustments in corn silage in the TMR were made as needed to maintain desired DM proportions of corn silage and concentrate. Corn silage and each batch mix of concentrate were sampled weekly and pooled by period, dried at 55°C, ground through a 2-mm screen and frozen (-10 °C) for later analyses. For whole cottonseed, Ca, Mg, Na, K and Cl were determined from composite samples by a commercial laboratory (Northeast DHIA Forage Testing Lab, Ithaca, NY). Nutrient content of corn silage and concentrate were analyzed by a commercial laboratory (Northeast DHIA Forage Testing Lab, Ithaca NY) for DM, CP, ADF, P, S, Fe, Zn, Cu, Mn,

and Mo. Energy concentration (NE_L) was calculated (Northeast DHIA Forage Testing Lab, Ithaca NY). Other analyses of concentrate and corn silage samples were done at the University of Florida Dairy Science Nutrition Lab. Concentrations of Ca, Mg, Na, K and Cl were determined from multiple analyses (n = 4 to 10) of pooled composite samples. Thawed feed samples were dried (100 °C for 24 h) and ashed (550 °C for 4 h). For analysis of Ca, Mg, Na, and K, feed samples were dissolved in 3N HCl, diluted with deionized H_2O and analyzed via atomic absorption spectrophotometry (Model 5000, Perkin Elmer, Inc., Norwalk, CT). For Cl analyses, feed samples were dissolved in 25 ml of .4N HNO_3 40% glacial acetic acid solution, shaken vigorously for 1 h and then determined via coulometric titration (Model 4-2500, Haake Buchler Instruments, Inc., Saddlebrook, NJ; Cotlove, 1963).

On the last morning of each period, after milking, but prior to feeding, jugular venous blood samples were drawn. Three ml were collected anaerobically into 5 ml plastic syringes coated with ammonium heparin (200 U/ml), capped, kept on ice, and analyzed for blood gases within 2 h of collection. Another 25 ml was taken into two 14 ml plastic syringes and decanted into two plastic tubes containing ammonium heparin (20 U/ml). One tube was centrifuged at 2500 x g for 20 minutes; plasma was harvested immediately, kept on ice for 4 h and frozen (-10 °C) for later mineral analyses. The other tube containing whole blood was frozen (-10 °C) for later mineral analyses. Blood pH, HCO_3^- and pCO_2 were determined from anaerobic samples on a Model 1304 pH/blood gas analyzer (Instrumentation Lab, Lexington, MA). Concentrations of plasma Ca, Mg, K and Na were analyzed after thawed samples were deproteinized

with 10% TCA, vortexed, centrifuged at 2500 x g for 10 min. Supernatant was harvested and diluted with deionized H_2O. Concentrations of Ca, Mg, K and Na in whole blood and milk were analyzed after digesting 1 ml of sample in 3 ml of concentrated HNO_3, heating in 30 ml glass tubes for 20 min and diluting with deionized water. Analysis of plasma, whole blood and milk samples for Ca, Mg, K and Na were by the same method as for feeds. Plasma, whole blood and milk were assayed for Cl using the same coulometric titration as for feeds; protein in whole blood and milk were precipitated with acid zinc sulfate prior to Cl analysis (somogyi precipitation; Cotlove, 1963).

Statistical Analysis

The mixtures design was used to explore responses to mixtures of $NaHCO_3$, NaCl, and KCl. Two separate statistical analyses were conducted: In the first, general linear models procedures of SAS (1985) were used to fit response surfaces. Sources of variation included cow and period as discrete variables and concentration of $NaHCO_3$, NaCl, and KCl in the salt mixture as continuous independent variables. The control diet had 0% of the mineral-salt mixture added to it, so to maintain symmetry of the design, control cow-period observations were excluded from this analysis. Mathematical equations for each dependent variable were generated from initial full-term statistical models consisting of: $Y = b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{123}x_1x_2x_3 + \epsilon$, where y equals the dependent variable, x_1 = $NaHCO_3$, x_2 = NaCl, x_3 = KCl, and ϵ = random error adjusted for effects of cow and period. Reduced models were established after fitting full-term models

and then removing terms that did not contribute ($P > .15$). Restraints on the regression procedure were established such that linear effects of $NaHCO_3$, NaCl and KCl always were included in models. Coefficients from reduced models were used to construct defined response surfaces. Surfaces were transformed into two-dimensional trace diagrams (Cornell, 1990) for presentation.

In the second analysis, general linear models procedures of SAS (1985) were used to evaluate treatment effects. Treatment effects were considered discrete. Comparisons of treatment effects were made by contrasts (Table 4-3). Each treatment had an average of 13 cow-period observations. Every treatment occurred with every other treatment at least three but no more than four times. No treatment followed any other treatment more than once and each cow received each treatment no more than once in the design. One cow was removed due to physical injury during period three; thus data from 107 cow-periods were included in the analysis. Because of unbalanced design and missing observations, least squares means were calculated. Standard errors are approximate.

Results

Tables 4-3 through 4-7 present least squares means and tests of contrasts for treatment effects of mixtures of $NaHCO_3$, NaCl and KCl on various responses. Figures 4-1 through 4-12 illustrate response surfaces plotted in two-dimensional trace diagrams. Figures 4-13 and 4-14 illustrate responses to CAD.

In the two-dimensional trace diagrams, points along the horizontal axis represent the percentages of each component in the mixture.

Regression curves are included for each component. The least squares mean for the control diet is plotted with the regression curves for comparison even though control cows were not included in the response surface analysis.

The effect of increasing each component from 0 to 100% in the mixture can be observed by moving from left to right along the horizontal axis. For example, the effect of 0% $NaHCO_3$ in the mixture is represented by the solid line at the 0:50:50 point on the horizontal axis. Because the mixture must always equal 100%, it contains 50% NaCl and 50% KCl at that point. By following the solid line from left to right, the response to increasing concentrations of $NaHCO_3$ in the mixture (and decreasing concentrations of NaCl and KCl) are observed. The response to 100% of each component is shown at the far right of each diagram.

Lactational Performance

Effects of different mixtures on measures of lactational performance are in Table 4-3. Mixtures of NaCl, KCl and $NaHCO_3$ in different proportions had no effects on DMI, MY, or milk fat percentage (MF) ($P > .1$; Table 4-3). Milk from cows fed the tertiary mixture (33% $NaHCO_3$: 33% NaCl: 33% KCl) had the lowest percentage protein ($P < .05$; Table 4-3). A large negative value for the b_{123} coefficient meant that the average response to $x_1:x_2:x_3$ (the tertiary mixture) differed from that of the binary and primary mixtures. For the milk protein percentage response this coefficient tended to be less ($P = .07$) than coefficients for other mixtures indicating that combining these three

TABLE 4-3. Effect of different mixtures of $NaHCO_3$, NaCl and KCl on dry matter intake (DMI), milk yield (MY), 3.5% fat-corrected milk (FCM) yield, milk fat percentage (MF), milk protein percentage (MP), and body weight gain (BWG).

	DMI (kg/d)	MY (kg/d)	3.5% FCM (kg/d)	MF (%)	MP (%)	BWG (kg/d)
Treatment[1] $NaHCO_3$:NaCl:KCl	----------Least Squares Means----------					
1, 100:0:0	21.9	26.4	26.6	3.54	3.18	.27
2, 0:100:0	22.0	26.9	26.7	3.51	3.22	.12
3, 0:0:100	21.4	26.6	26.1	3.44	3.12	.67
4, 50:50:0	22.0	26.9	26.6	3.47	3.17	.43
5, 50:0:50	21.7	26.5	26.2	3.46	3.18	.09
6, 0:50:50	21.6	26.6	26.3	3.45	3.17	.33
7, 33:33:33	21.6	27.4	27.3	3.51	3.09	.24
8, 0:0:0 (Control)	21.4	26.3	26.3	3.54	3.21	.15
SEM	.30	.53	.69	.11	.04	.15
Contrasts	----------Probability Values----------					
Treatment 1 - 7 vs. 8	NS[a]	NS	NS	NS	NS	NS
Treatment 1 - 6 vs. 7	NS	NS	NS	NS	.03	NS
Treatment 4,5 vs. 7	NS	NS	NS	NS	.05	NS
Treatment 2,3,6 vs. 1,4,5	NS	NS	NS	NS	NS	NS
Treatment 2,3 vs. 6	NS	NS	NS	NS	NS	NS
Treatment 2 vs. 3	NS	NS	NS	NS	.07	.02
Treatment 4 vs. 5	NS	NS	NS	NS	NS	NS

[1]Relative proportion of $NaHCO_3$, NaCl and KCl in the mineral mixture.

[a]$P > .10$.

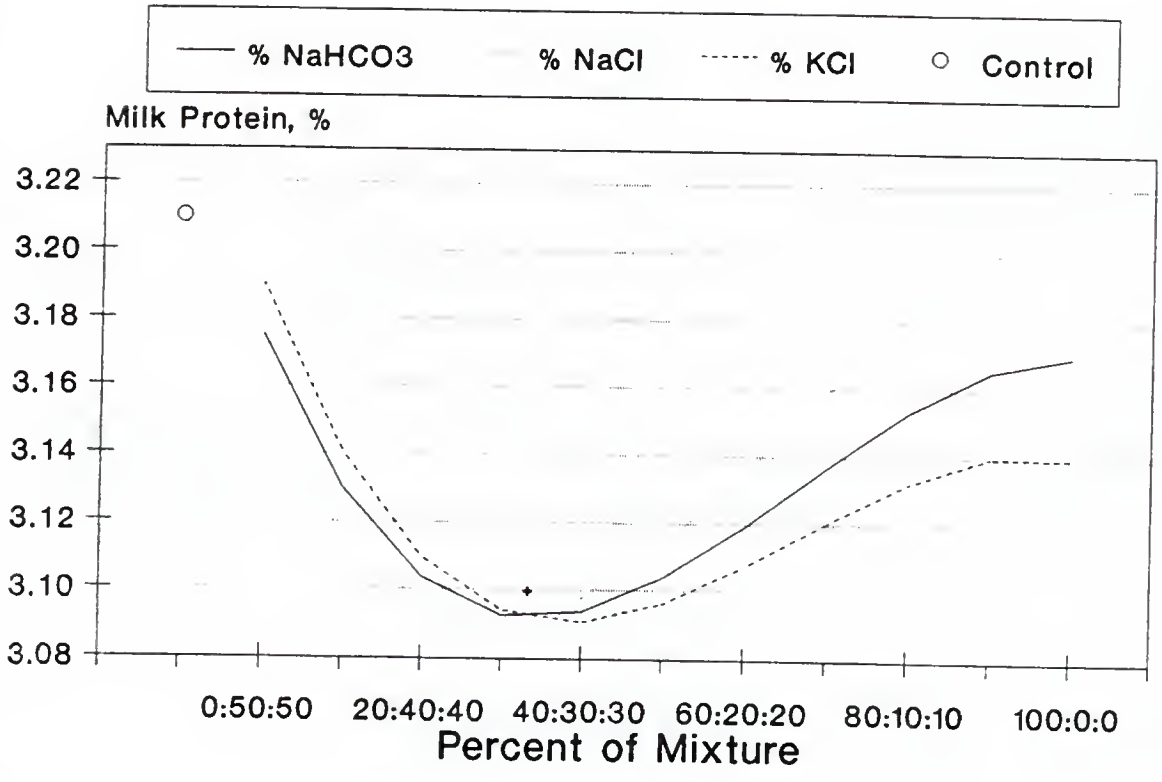

Figure 4-1. Regression of milk protein (MP, %) response to binary (0:50:50), tertiary (33:33:33) and primary (100:0:0) mixtures of $NaHCO_3$ (—), NaCl (•••) and KCl (---). Control diet (open circle) not included in regression but shown for visual comparison. Points along the horizontal axis represent the percentage of each component in the mixture. Regression for MP with SE of coefficients in parentheses: MP = 3.17 x $NaHCO_3$ (.04) + 3.22 x NaCl (.04) + 3.14 x KCl (.04) - 2.20 x $NaHCO_3$ x NaCl x KCl (1.08)$^{+}$. SEM = .04. $^{+}P < .10$.

components depressed milk protein percentage (Figure 4-1). Milk from cows fed the mixture with 100% NaCl had a higher concentration of protein ($P = .07$) than milk from cows fed the 100% KCl mixture. Cows fed the 100% KCl mixture gained more ($P < .05$) than cows fed the 100% NaCl mixture (Table 4-3; Figure 4-2). Response surface analysis revealed that cows fed the mineral mixture containing 50% $NaHCO_3$ and 50% KCl gained less than cows fed other binary mixtures ($P < .05$; Table 4-3; Figure 4-2).

Mineral Metabolism

Effects of different mixtures on plasma mineral concentrations are in Table 4-4. Plasma Na was highest ($P = .06$) in control cows. Plasma K tended to be lower for cows fed the binary $NaHCO_3$ mixtures as compared with those fed the tertiary mixture ($P = .08$). Plasma K was higher ($P < .05$) for cows fed the 50% $NaHCO_3$:50% NaCl mixture than for those fed the 50% $NaHCO_3$:50% KCl mixture. Response surface analysis indicated that the tertiary mixture lowered ($P < .05$) PK and the binary $NaHCO_3$ and NaCl mixture increased ($P < .05$) PK (Figure 4-3). Plasma Cl was higher ($P < .05$) for cows fed the 50:50 mixture of NaCl and KCl as compared with the pooled average of those fed 100% NaCl and 100% KCl (Figure 4-4). Plasma Ca tended to be lower ($P = .09$) for cows fed the 100% KCl than for those fed the 100% NaCl mixture. Plasma Ca was reduced by feeding the 100% KCl mineral mixture ($P < .05$; Figure 4-5) compared with 100% $NaHCO_3$ or 100% NaCl in the mixture. There was a tendency for lower ($P = .07$) PMg in cows fed the 50:50 mixture of NaCl and KCl as compared to the mean PMg from cows fed either 100% NaCl or 100% KCl in the mixture. Plasma

TABLE 4-4. Effect of different mixtures of $NaHCO_3$, NaCl and KCl on blood plasma Na (PNa), K (PK), Cl (PCl), Ca (PCa) and Mg (PMg).

	PNa (meq/L)	PK (meq/L)	PCl (meq/L)	PCa (meq/L)	PMg (meq/L)
Treatment[1] $NaHCO_3$:NaCl:KCl	----------Least Squares Means----------				
1, 100:0:0	128.68	5.81	98.77	4.80	1.78
2, 0:100:0	127.85	5.61	97.43	4.76	1.78
3, 0:0:100	128.61	5.82	98.53	4.63	1.88
4, 50:50:0	128.85	5.98	98.94	4.71	1.78
5, 50:0:50	127.90	5.67	99.06	4.70	1.78
6, 0:50:50	127.96	5.69	100.29	4.70	1.74
7, 33:33:33	128.79	5.62	99.23	4.75	1.80
8, 0:0:0 (control)	129.95	5.85	98.67	4.78	1.79
SEM	.80	.10	.77	.06	.04
Contrasts	----------Probability Values----------				
Treatment 1 - 7 vs. 8	.06	NS[a]	NS	NS	NS
Treatment 1 - 6 vs. 7	NS	NS	NS	NS	NS
Treatment 4,5 vs. 7	NS	.08	NS	NS	NS
Treatment 2,3,6 vs. 1,4,5	NS	NS	NS	NS	NS
Treatment 2,3 vs. 6	NS	NS	.02	NS	.07
Treatment 2 vs. 3	NS	NS	NS	.09	.09
Treatment 4 vs. 5	NS	.03	NS	NS	NS

[1]Relative proportion of $NaHCO_3$, NaCl and KCl in the mineral mixture.

[a]$P > .10$.

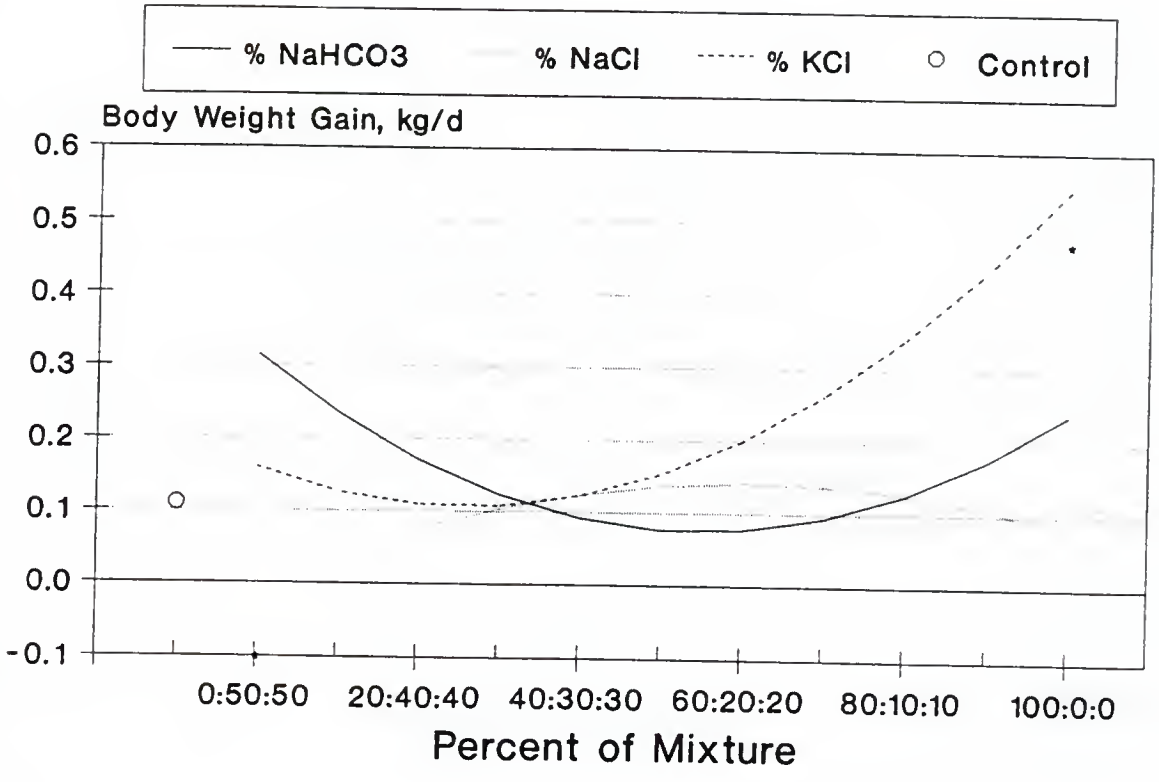

Figure 4-2. Regression of body weight gain (BWG, kg/d) response to binary (0:50:50), tertiary (33:33:33) and primary (100:0:0) mixtures of $NaHCO_3$ (—), NaCl (•••) and KCl (---). Control diet (open circle) not included in regression but shown for visual comparison. Points along the horizontal axis represent the percentage of each component in the mixture. Regression for BWG with SE of coefficients in parentheses: BWG = .24 x $NaHCO_3$ (.20) + .08 x NaCl (.20) + .55 x KCl (.20)* - 1.61 x $NaHCO_3$ x KCl (.71)*. SEM = .15. *P < .05.

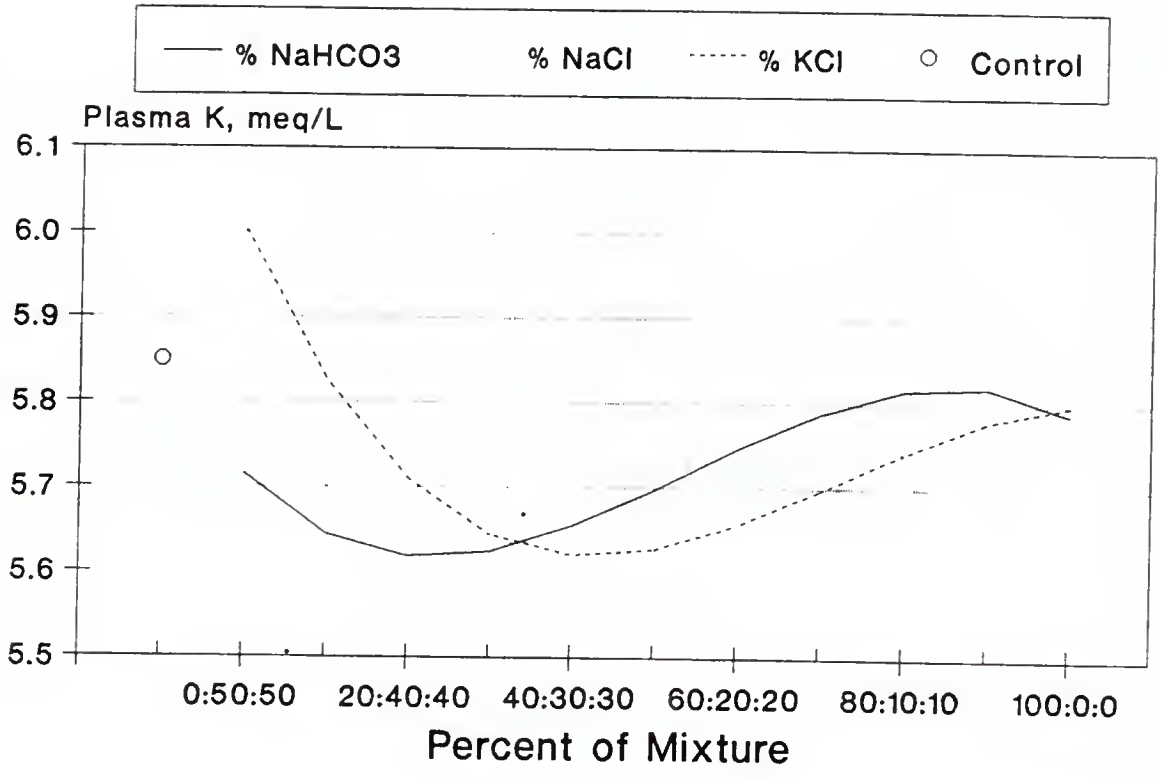

Figure 4-3. Regression of plasma K (PK, meq/L) response to binary (0:50:50), tertiary (33:33:33) and primary (100:0:0) mixtures of $NaHCO_3$ (—), NaCl (•••) and KCl (---). Control diet (open circle) not included in regression but shown for visual comparison. Points along the horizontal axis represent the percentage of each component in the mixture. Regression for PK with SE of coefficients in parentheses: PK = 5.79 x $NaHCO_3$ (.10) + 5.63 x NaCl (.10) + 5.80 x KCl (.10) + 1.17 x $NaHCO_3$ x NaCl (.51)* - 6.40 x $NaHCO_3$ x NaCl x KCl (2.95)*. SEM = .10. *$P < .05$.

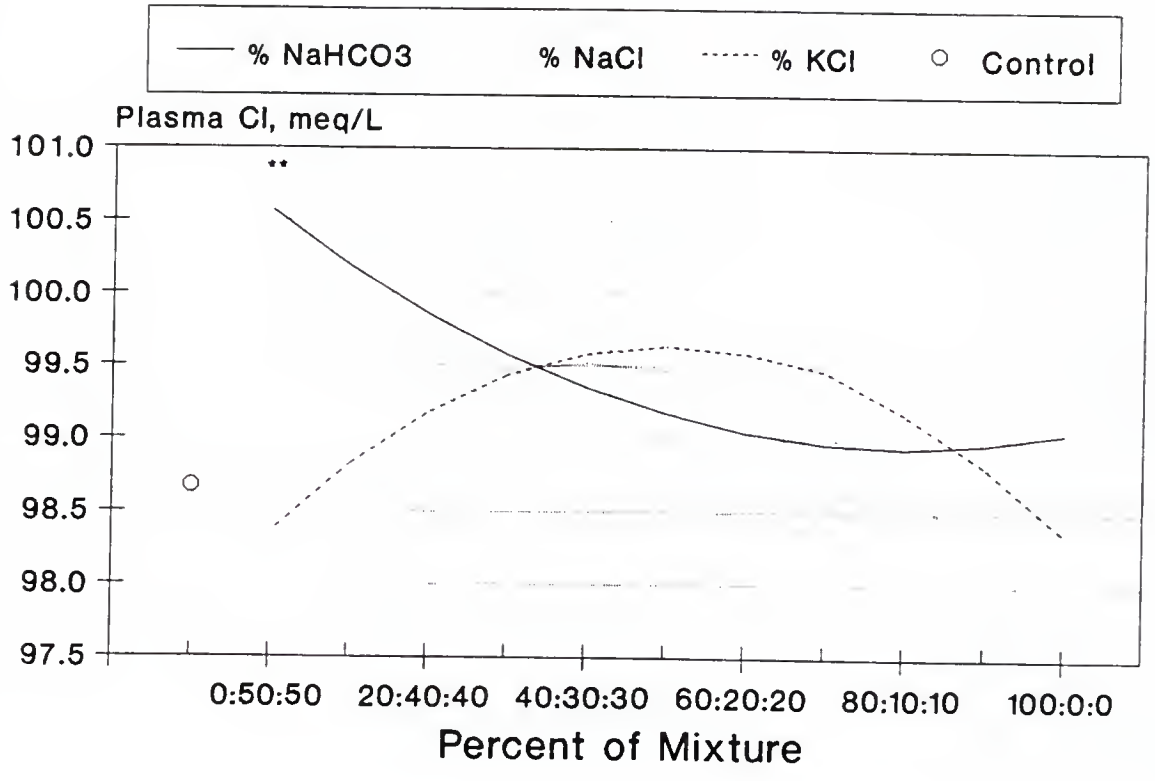

Figure 4-4. Regression of plasma Cl (PCl, meq/L) response to binary (0:50:50), tertiary (33:33:33) and primary (100:0:0) mixtures of $NaHCO_3$ (—), NaCl (•••) and KCl (---). Control diet (open circle) not included in regression but shown for visual comparison. Points along the horizontal axis represent the percentage of each component in the mixture. Regression for PCl with SE of coefficients in parentheses: PCl = 99.05 x $NaHCO_3$ (.76) + 97.66 x NaCl (.73) + 98.36 x KCl (.75) + 10.24 x NaCl x KCl (3.49)**. SEM = .77. **P < .01.

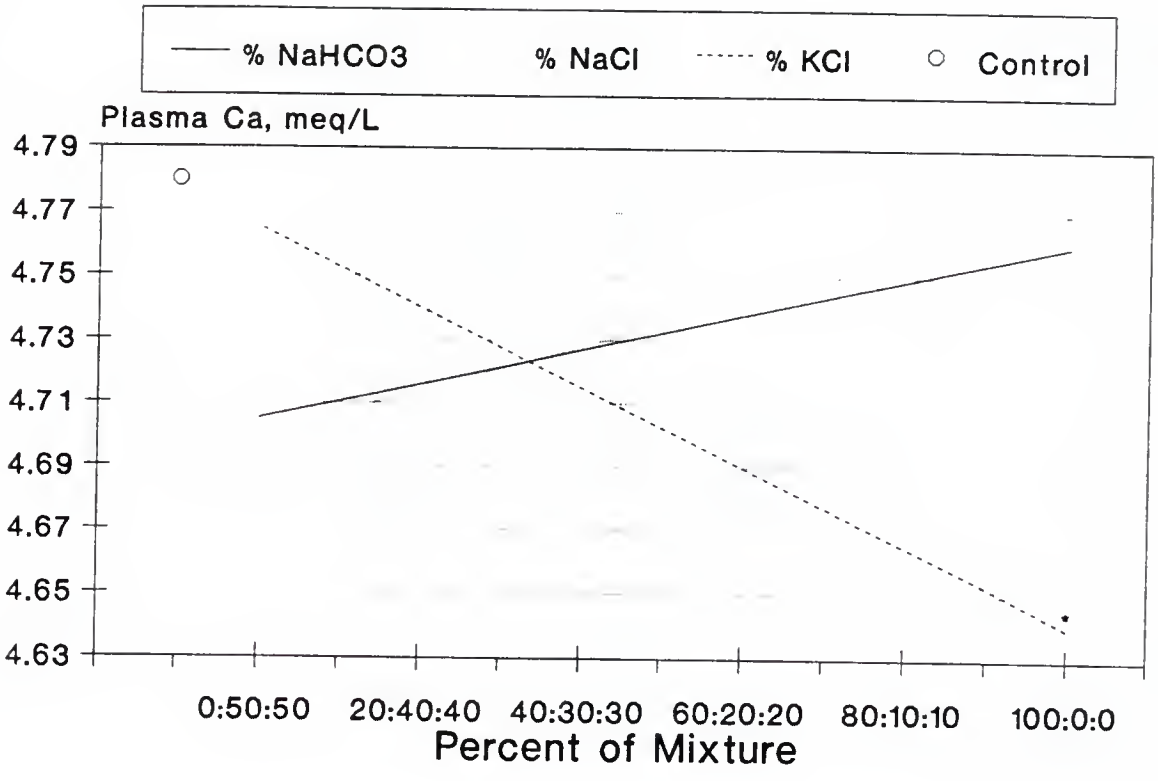

Figure 4-5. Regression of plasma Ca (PCa, meq/L) response to binary (0:50:50), tertiary (33:33:33) and primary (100:0:0) mixtures of $NaHCO_3$ (—), NaCl (•••) and KCl (---). Control diet (open circle) not included in regression but shown for visual comparison. Points along the horizontal axis represent the percentage of each component in the mixture. Regression for PCa with SE of coefficients in parentheses: PCa = 4.76 x $NaHCO_3$ (.06) + 4.77 x NaCl (.06) + 4.64 x KCl (.06)*. SEM = .06. *P < .05.

Mg concentrations tended to be greater (P = .09) from cows fed the 100% KCl than from those fed the 100% NaCl mixture. Differences in PMg were not detected in response surface analysis.

Effects of different mixtures on whole blood mineral concentrations are in listed in Table 4-5. Whole blood K was lower (P < .03) and whole blood Na tended to be lower (P = .10) with 100% NaCl than with 100% KCl (Table 5; Figure 4-6). In contrast, whole blood Cl was higher (P < .05) with 100% NaCl than with 100% KCl. Response surface analysis also demonstrated that whole blood K concentration for cows fed 100% NaCl was lower (P < .05) than in cows fed 100% $NaHCO_3$ and 100% KCl. The coefficient estimate for the $NaHCO_3$:KCl term tended to be more negative (P < .10) than coefficients for other binary mixture terms indicating that the $NaHCO_3$:KCl mixture may have had an antagonistic effect on whole blood K concentrations (Figure 4-6). Following the plasma trend, whole blood Cl from cows fed the 50:50 NaCl:KCl mixture was greater (P < .05) than from cows fed 100% NaCl and 100% KCl mixtures (pooled together; Table 4-5). The 100% NaCl mixture increased WBCl (P < .05) whereas the 100% KCl mixture tended to reduce WBCl (P ≤ .10) (Figure 4-7).

Effects of different mixtures on milk mineral concentrations are in Table 4-6. Milk Na tended to be lowest from cows fed the tertiary mixture (P = .1; Table 4-6; Figure 4-8). Milk Na tended to be greater (P = .1) from cows fed the 100% NaCl mixture than from cows fed the 100% KCl mixture. Milk Na tended to be greater (P = .09) from cows fed the 50% $NaHCO_3$:50% NaCl mixture than from cows fed the 50% $NaHCO_3$:50% KCl mixture (Table 4-6; Figure 4-8). Milk K concentrations were lower

TABLE 4-5. Effect of different mixtures of $NaHCO_3$, NaCl and KCl on whole blood Na (WBNa), K (WBK), Cl (WBCl), Ca (WBCa), and Mg (WBMg).

	WBNa (meq/L)	WBK (meq/L)	WBCl (meq/L)	WBCa (meq/L)	WBMg (meq/L)
Treatment[1] $NaHCO_3$:NaCl:KCl	----------Least Squares Means----------				
1, 100:0:0	90.85	10.45	80.84	2.32	1.69
2, 0:100:0	89.10	10.01	83.61	2.34	1.64
3, 0:0:100	92.23	10.71	80.36	2.31	1.71
4, 50:50:0	91.45	10.30	84.52	2.35	1.66
5, 50:0:50	89.00	10.13	82.93	2.29	1.61
6, 0:50:50	89.85	9.98	84.71	2.40	1.63
7, 33:33:33	91.21	10.15	82.86	2.33	1.67
8, 0:0:0 (control)	90.60	10.11	83.21	2.25	1.66
SEM	1.32	.22	1.07	.05	.04
Contrasts	----------Probability Values----------				
Treatment 1 - 7 vs. 8	NS[a]	NS	NS	NS	NS
Treatment 1 - 6 vs. 7	NS	NS	NS	NS	NS
Treatment 4,5 vs. 7	NS	NS	NS	NS	NS
Treatment 2,3,6 vs. 1,4,5	NS	NS	NS	NS	NS
Treatment 2,3 vs. 6	NS	NS	.04	NS	NS
Treatment 2 vs. 3	.10	.03	.04	NS	NS
Treatment 4 vs. 5	NS	NS	NS	NS	NS

[1]Relative proportion of $NaHCO_3$, NaCl and KCl in the mineral mixture.

[a]$P > .10$.

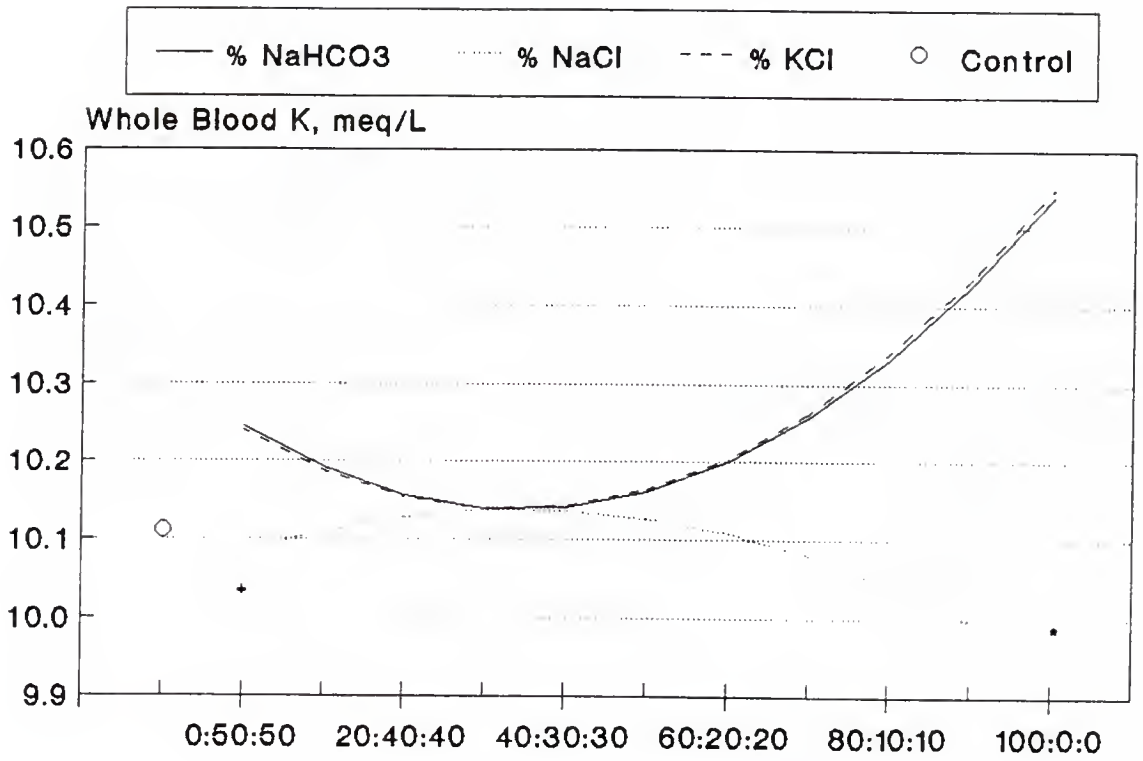

Figure 4-6. Regression of whole blood K (WBK, meq/L) response to binary (0:50:50), tertiary (33:33:33) and primary (100:0:0) mixtures of $NaHCO_3$ (—), NaCl (•••) and KCl (---). Control diet (open circle) not included in regression but shown for visual comparison. Points along the horizontal axis represent the percentage of each component in the mixture. Regression for WBK with SE of coefficients in parentheses = 10.54 x $NaHCO_3$ (.23) + 9.94 x NaCl (.22)* + 10.55 x KCl (.23) - 1.85 x NaCl x KCl (1.05)$^{+}$. SEM = .22. $^{*}P < .05$; $^{+}P < .10$.

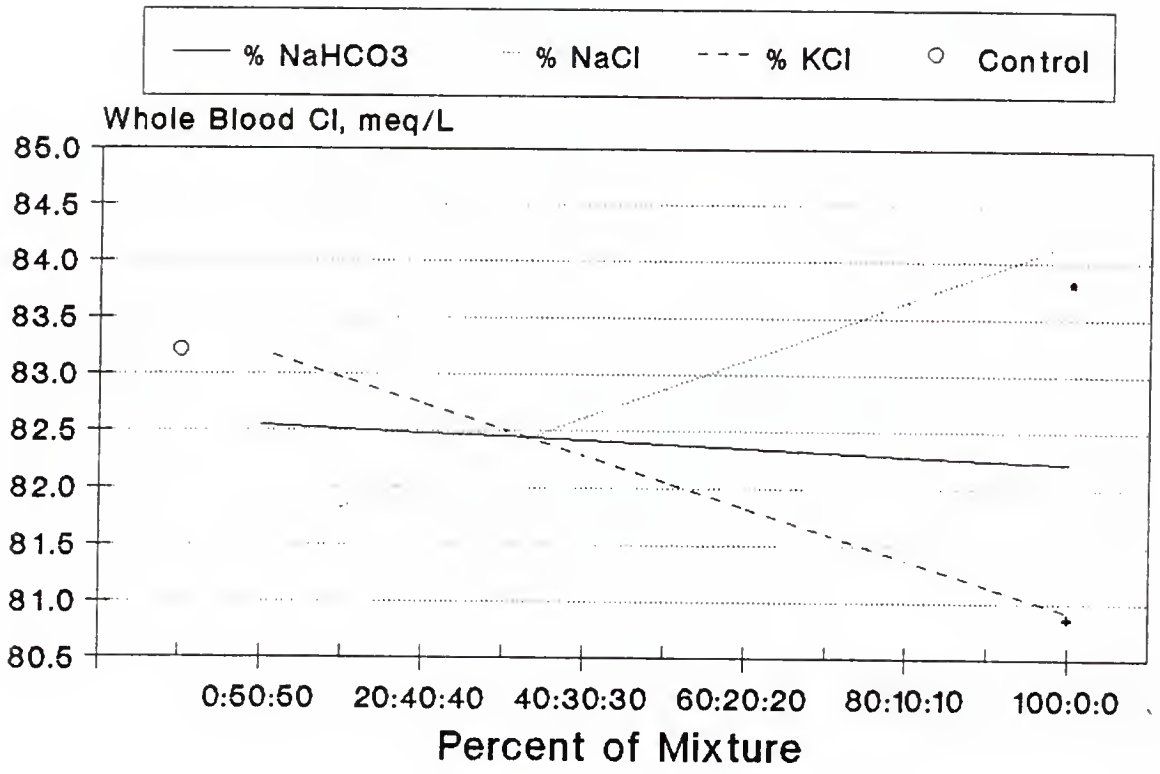

Figure 4-7. Regression of whole blood Cl (WBCl, meq/L) response to binary (0:50:50), tertiary (33:33:33) and primary (100:0:0) mixtures of $NaHCO_3$ (—), NaCl (•••) and KCl (---). Control diet (open circle) not included in regression but shown for visual comparison. Points along the horizontal axis represent the percentage of each component in the mixture. Regression for WBCl with SE of coefficients in parentheses: WBCl = 82.22 x $NaHCO_3$ (1.07) + 84.17 x NaCl (1.07)* + 80.92 x KCl (1.07)$^{+}$. SEM = 1.07. $^{*}P < .05$; $^{+}P < .10$.

TABLE 4-6. Effect of different mixtures of $NaHCO_3$, NaCl and KCl on milk Na (MLNa), K (MLK), Cl (MLCl), Ca (MLCa) and Mg (MLMg).

	MLNa (meq/L)	MLK (meq/L)	MLCl (meq/L)	MLCa (meq/L)	MLMg (meq/L)
Treatment[1] $NaHCO_3$:NaCl:KCl	----------Least Squares Means----------				
1, 100:0:0	17.85	36.60	25.87	45.95	8.00
2, 0:100:0	18.76	37.73	26.98	45.87	8.21
3, 0:0:100	17.25	38.05	27.03	46.32	8.18
4, 50:50:0	19.81	36.62	28.55	48.68	8.12
5, 50:50:0	18.18	37.45	26.50	46.01	8.23
6, 0:50:50	17.96	36.74	26.00	47.85	8.36
7, 33:33:33	17.15	36.80	26.77	45.42	8.06
8, 0:0:0 (control)	18.51	35.52	25.82	47.86	8.45
SEM	.64	.60	.88	1.06	.14
Contrasts	----------Probability Values----------				
Treatment 1 - 7 vs. 8	NS[a]	.01	NS	NS	.06
Treatment 1 - 6 vs. 7	.10	NS	NS	NS	NS
Treatment 4,5 vs. 7	.02	NS	NS	NS	NS
Treatment 2,3,6 vs. 1,4,5	NS	NS	NS	NS	NS
Treatment 2,3 vs. 6	NS	NS	NS	NS	NS
Treatment 2 vs. 3	.10	NS	NS	NS	NS
Treatment 4 vs. 5	.09	NS	NS	.09	NS

[1]Relative proportion of $NaHCO_3$, NaCl and KCl in the mineral mixture.

[a]$P > .10$.

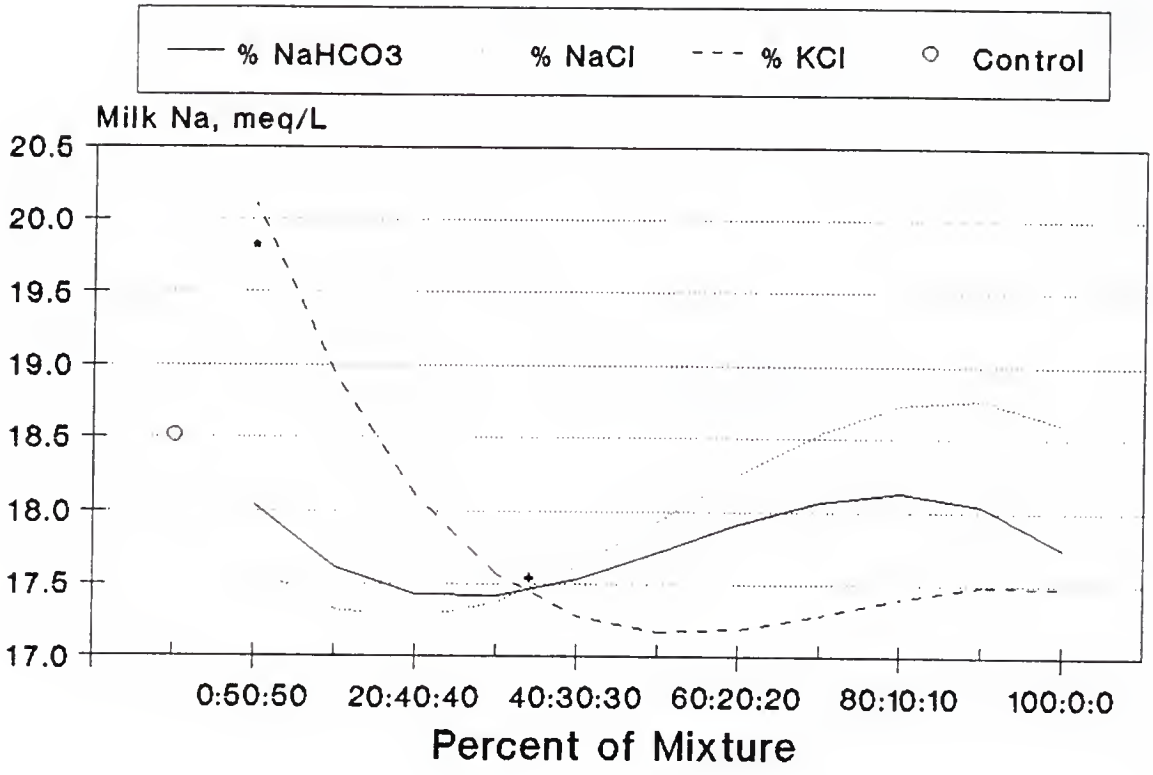

Figure 4-8. Regression of milk Na (MLNa, meq/L) response to binary (0:50:50), tertiary (33:33:33) and primary (100:0:0) mixtures of $NaHCO_3$ (—), NaCl (•••) and KCl (---). Control diet (open circle) not included in regression but shown for visual comparison. Points along the horizontal axis represent the percentage of each component in the mixture. Regression for MLNa with SE of coefficients in parentheses: MLNa = 17.75 x $NaHCO_3$ (.66) + 18.60 x NaCl (.64) + 17.48 x KCl (.66) + 7.71 x $NaHCO_3$ x NaCl $(3.30)^*$ - 36.52 x $NaHCO_3$ x NaCl x KCl $(19.22)^+$. SEM = .64. $^*P < .05$; $^+P < .10$.

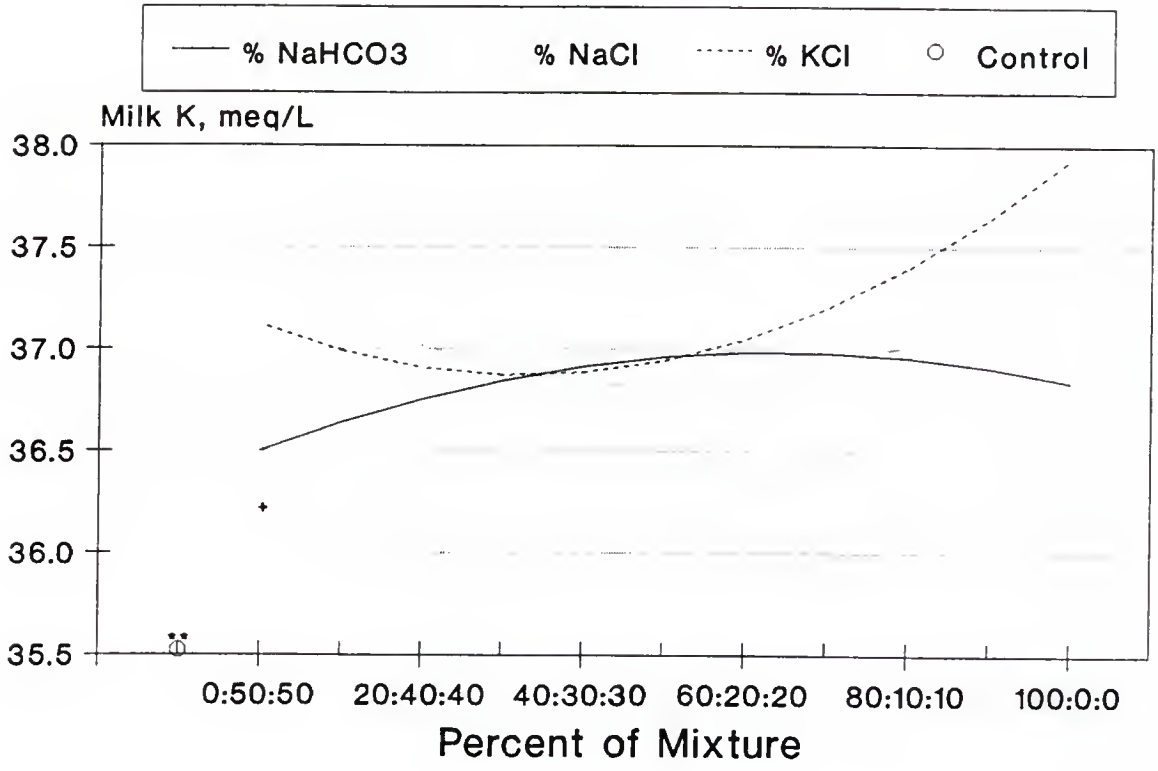

Figure 4-9. Regression of milk K (MLK, meq/L) response to binary (0:50:50), tertiary (33:33:33) and primary (100:0:0) mixtures of $NaHCO_3$ (—), NaCl (•••) and KCl (---). Control diet (open circle) not included in regression but shown for visual comparison. Points along the horizontal axis represent the percentage of each component in the mixture. Regression for MLK with SE of coefficients in parentheses: MLK = 36.84 x $NaHCO_3$ (.59) + 37.40 x NaCl (.57) + 37.94 x KCl (.57) - 4.68 x NaCl x KCl (2.69)$^{+}$. SEM = .60. $^{**}P < .01$; $^{+}P < .10$.

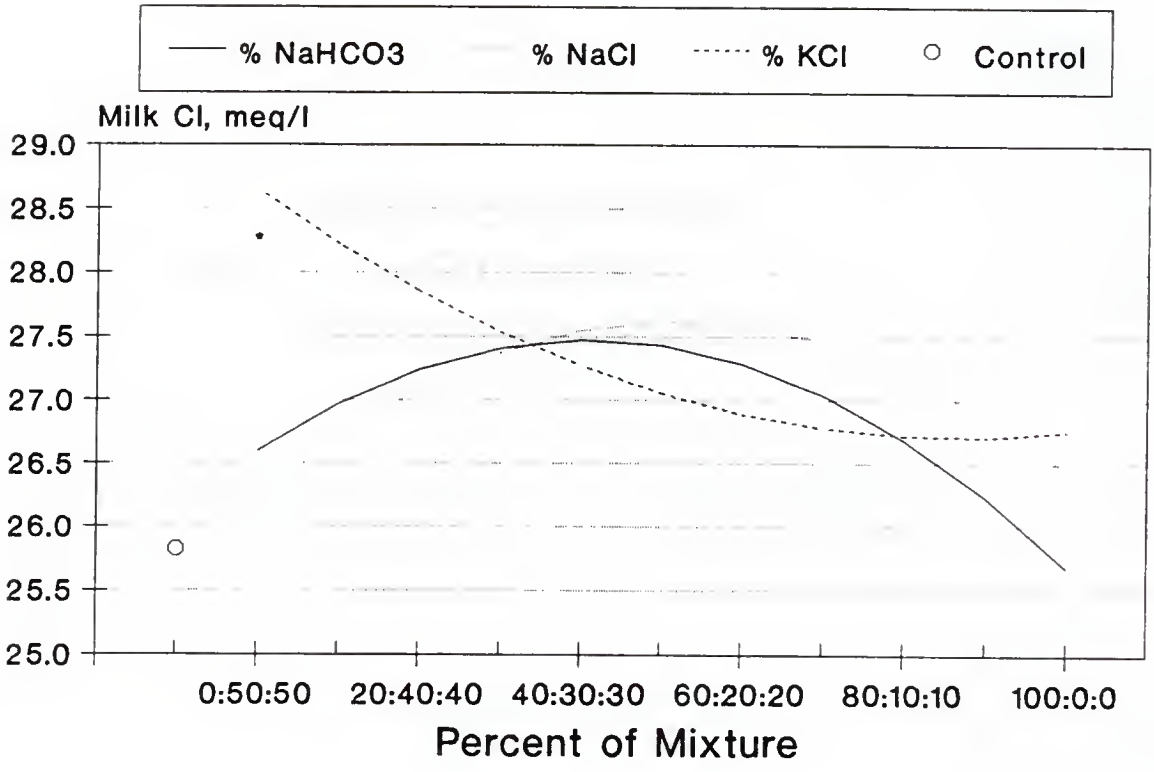

Figure 4-10. Regression of milk Cl (MLCl, meq/L) response to binary (0:50:50), tertiary (33:33:33) and primary (100:0:0) mixtures of $NaHCO_3$ (—), NaCl (•••) and KCl (---). Control diet (open circle) not included in regression but shown for visual comparison. Points along the horizontal axis represent the percentage of each component in the mixture. Regression for MLCl with SE of coefficients in parentheses: MLCl = 25.68 x $NaHCO_3$ (.89) + 26.44 x NaCl (.86) + 26.76 x KCl (.89) + 10.33 x $NaHCO_3$ x NaCl (4.27)*. SEM = .88. *P < .05.

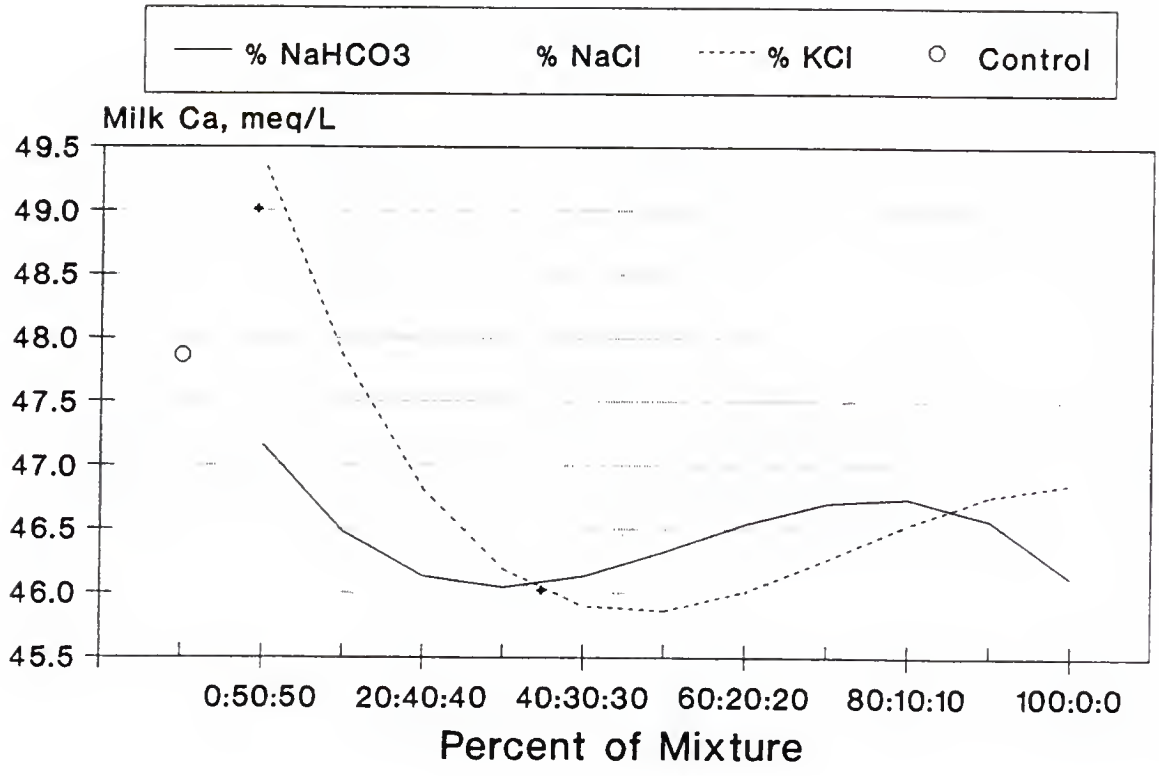

Figure 4-11. Regression of milk Ca (MLCa, meq/L) response to binary (0:50:50), tertiary (33:33:33) and primary (100:0:0) mixtures of $NaHCO_3$ (—), NaCl (•••) and KCl (---). Control diet (open circle) not included in regression but shown for visual comparison. Points along the horizontal axis represent the percentage of each component in the mixture. Regression for MLCa with SE of coefficients in parentheses: MLCa = 46.12 x $NaHCO_3$ (1.09) + 47.47 x NaCl (1.05) + 46.86 x KCl (1.08) + 10.33 x $NaHCO_3$ x NaCl (5.35)$^{+}$ - 51.54 x $NaHCO_3$ x NaCl x KCl (31.18). SEM = 1.06. $^{+}P < .10$.

TABLE 4-7. Effect of different mixtures of $NaHCO_3$, NaCl and KCl on whole blood hydrogen ion concentration (H^+), bicarbonate (HCO_3^-), pCO_2, anion gap (ANGAP) and base excess (BE). Values for whole blood pH are shown for reference only.

	H^+ (neq/L)	pH	HCO_3^- (meq/L)	pCO_2 (mm Hg)	ANGAP[2] (meq/L)	BE (meq/L)
Treatment[1] $NaHCO_3$:NaCl:KCl	----------Least Squares Means----------					
1, 100:0:0	48.09	7.320	26.26	50.58	9.48	.00
2, 0:100:0	47.15	7.329	26.55	50.19	9.50	.50
3, 0:0:100	49.65	7.308	26.22	52.00	9.71	-.28
4, 50:50:0	47.71	7.324	26.44	50.81	9.47	.23
5, 50:0:50	48.05	7.320	25.98	49.90	8.54	-.22
6, 0:50:50	47.57	7.325	26.04	49.60	7.52	-.06
7, 33:33:33	48.33	7.317	25.04	48.37	10.08	-1.06
8, 0:0:0 (control)	46.91	7.335	26.70	49.93	10.39	.69
SEM	1.47		.52	1.37	.84	.60
Contrasts	----------Probability Values----------					
Treatment 1 - 7 vs. 8	NS[a]		NS	NS	NS	NS
Treatment 1 - 6 vs. 7	NS		.03	NS	NS	.10
Treatment 4,5 vs. 7	NS		.07	NS	NS	NS
Treatment 2,3,6 vs. 1,4,5	NS		NS	NS	NS	NS
Treatment 2,3 vs. 6	NS		NS	NS	.06	NS
Treatment 2 vs. 3	NS		NS	NS	NS	NS
Treatment 4 vs. 5	NS		NS	NS	NS	NS

[1]Relative proportion of $NaHCO_3$, NaCl and KCl in the mineral mixture.

[2]Anion gap calculated as meq $[(Na + K) - (Cl + HCO_3^-)]$/L whole blood.

[a]$P > .10$.

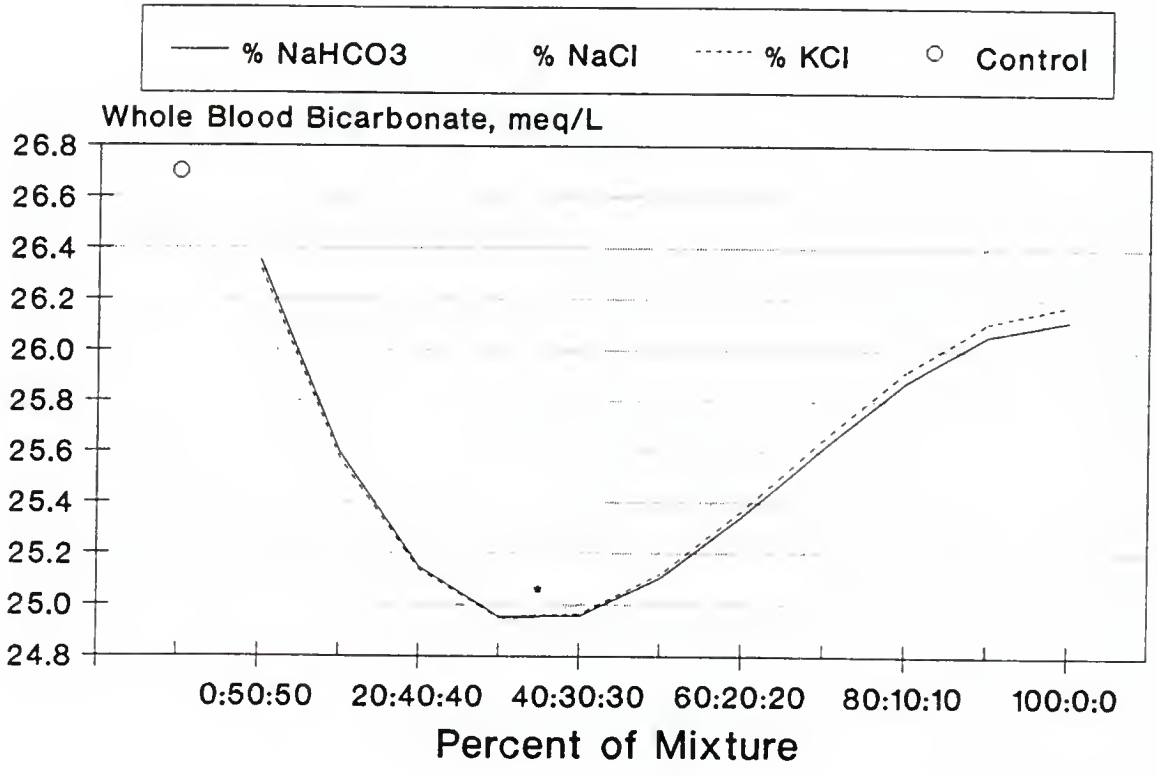

Figure 4-12. Regression of whole blood bicarbonate (HCO_3^-, meq/L) response to binary (0:50:50), tertiary (33:33:33) and primary (100:0:0) mixtures of $NaHCO_3$ (—), NaCl (•••) and KCl (---). Control diet (open circle) not included in regression but shown for visual comparison. Points along the horizontal axis represent the percentage of each component in the mixture. Regression for HCO_3^- with SE of coefficients in parentheses: HCO_3^- = 26.12 x $NaHCO_3$ (.57) + 26.53 x NaCl (.55) + 26.18 x KCl (.57) - 36.22 x $NaHCO_3$ x NaCl x KCl (16.48)*. SEM = .52. *$P < .05$.

(P = .01) in control than in treatment groups. Milk Ca tended to be greatest (P < .10) for cows fed the 50% $NaHCO_3$:50% NaCl mixture, and was lowest from those fed the tertiary mixture (P = .06; Figure 4-11). The 50% $NaHCO_3$:50% NaCl fed group had greater MLCa (P=.06; Figure 11) than those fed 50% $NaHCO_3$:50% KCl. Milk Mg was highest (P=.06) in control cows and was higher (P < .05) in all primary and binary mixtures, as well as binary $NaHCO_3$ mixtures pooled together, compared with the cows fed the tertiary mixture. Milk Mg was greater from cows fed 100% NaCl vs. 100% KCl. Differences in MLMg were not detected in response surface analysis.

Acid-Base Status

Effects of different mixtures on measures of acid-base status are in Table 4-7. Plasma anion gap tended to be lower (P = .06) with the 50:50 mixture of NaCl and KCl than with 100% NaCl and 100% KCl. Blood HCO_3^- was lowest (P < .05; Figure 4-12) and blood base excess tended to be lowest (P = .10) in cows fed the tertiary mixture. No other differences in acid-base status were noted (P > . 10).

Discussion

Mixtures designs are employed to study the response to mixtures of components in an attempt to see if there are mixtures that are more effective than any of the single components used separately. Although mixtures designs have been used infrequently in dairy cattle nutrition, these designs are an effective tool to study responses to mixtures of feed ingredients (Papas et al., 1984). In the three-component mixtures

design used in this study, tertiary, binary and primary mixtures of $NaHCO_3$, NaCl and KCl on various lactational and physiological responses were evaluated. Synergistic and antagonistic effects of these salts (in a 1% DM addition to a basal diet, already adequate in the nutrients supplied by these salts) were evaluated. Although only seven mixtures were used, the response surface analysis generated regression equations which could be used to predict the effect of any combination of $NaHCO_3$, NaCl and KCl. Also, this mixtures design was useful for comparisons of the response to the individual salts.

The discovery that KCl depressed milk protein percentage is not without precedence. Increasing dietary K and Cl depressed milk protein percentage in other studies. Pradhan and Hemken (1968) noted a decrease in concentration of protein in milk when dietary K increased from deficient concentrations to 1.8%. Escobosa et al. (1984) reported reduced milk protein percentage in cows fed 2.28% $CaCl_2$. West et al. (1987) using KCl and K_2CO_3 reported a decline in milk protein percentage with decreasing Cl (from .77 to .46%) when dietary K was held constant at 1.4%. Increasing either dietary K or Cl reduced milk protein percentage (Sanchez et al., 1990a) (chapter 3 in this dissertation). Although an effect of K and/or Cl on milk protein has been observed prior to this study, no hypothesis has been advanced to explain the mechanism.

The other mineral salt mixture that depressed milk protein in the current study was the tertiary mixture containing 33% of each component. The cause for this effect is unknown but may be related to changes in acid-base status and mineral metabolism. The tertiary and 100% KCl

mixture affected milk protein similarly to the way these mixtures affected acid-base variables such as blood HCO_3^-, base-excess and Cl in whole blood. Tucker et al. (1988a) found differences in blood acid-base status that were correlated with alterations in ruminal fermentation. Changes in ruminal fermentation could have been a direct result of changes in acid-base balance. Apparently, the association between milk protein synthesis and acid-base and mineral metabolism has not been studied. Influence of KCl, alone or in combination with $NaHCO_3$ and NaCl, on milk protein synthesis is not clear but warrants further study.

There was a difference between the control treatment and the mineral mixtures in this study. The control-fed cows had greater concentration of Na in the plasma. Mineral-salt mixtures may have increased plasma volume and led to a dilution of Na in plasma. However, plasma volume measures were not made so this cannot be confirmed. Concentration of Na in plasma normally is regulated tightly, but because plasma K and Cl of cows fed the control diet followed the same trend (although nonsignificant) as plasma Na, a dilution of the plasma resulting from the mineral salts was a plausible explanation. Plasma osmolality and blood volume are dependent upon Na concentrations more than any other osmotic determinant (Lunn and McGuirk, 1990). When excess or deficient amounts of Na are absorbed, extracellular water concentration usually increases or decreases to maintain relatively constant Na concentration (Kleinman and Lorenz, 1989). Feeding $NaHCO_3$ generally does not change plasma Na concentrations (Kilmer et al., 1981; Erdman et al., 1982; Schneider et al., 1984b).

Plasma K was higher in cows fed the 50% $NaHCO_3$:50% NaCl mixture than in those fed the 50% $NaHCO_3$:50% KCl. Cows consuming a diet supplemented with a multielement buffer consisting of a mixture of Na, K, Cl, Mg, and S salts had lower plasma K as compared with those fed a diet with either $NaHCO_3$ or no buffer (Staples et al., 1988). Plasma Cl was increased for cows fed the 50:50 mixture of NaCl and KCl. Increase PCl for cows fed the 50:50 mixture of NaCl and KCl also was evident in the response surface analysis, but the reason that these salts fed individually did not elevate PCl is unknown. Plasma Ca tended to be lower in cows fed the 100% KCl than in cows fed 100% NaCl mineral mixtures. Plasma Ca is known to be related to acid-base status (Oetzel et al., 1988; Wang and Beede, 1992) indicating that differences between NaCl and KCl may be related to acid-base status.

The differences between 100% NaCl and 100% KCl treatments on whole blood Na, K and Cl may have reflected differences in red blood cell concentrations of these minerals. Plasma Na, K and Cl responses were not significant for the 100% NaCl vs. 100% KCl contrast which suggests that concentration differences in whole blood were due to concentration differences in red blood cells.

An involvement of the mammary gland in mineral homeostasis was evident in this study. As Coppock (1982a) suggested, the obligatory demand for minerals needed for milk synthesis by the lactating mammary gland may have facilitated homeostatic mechanisms to differences in dietary intake.

Response to CAD

To account for potential interrelationships among dietary Na, K and Cl, researchers have attempted to relate acid-base status and lactational performance to a linear combination of these three minerals. The basic theory behind this concept is that body fluids must remain electrically neutral and because absorbed, non metabolized ions (such as Na, K and Cl) contribute positive and negative charges to the system, they affect electrical balance of the body. This affects acid-base status which can in turn influence lactational performance. From work with poultry and swine, it was determined that Na, K and Cl were the most important elements of this expression (Mongin, 1980).

This concept was first evaluated with lactating dairy cattle by Tucker et al. (1988a) who compared diets with -10, 0, +10 and +20 CAD. A diet with a sum of +20 meq improved DMI by 11% and MY by 9% compared with a diet with -10 meq, independent of the individual minerals (Na, K or Cl) used to vary CAD. Blood, rumen, and urine measures indicated an improvement in acid-base status with high the cation (+20 meq) diet as compared to low cation (-10 meq) diet. It was hypothesized that dietary CAD may become a useful tool for improving the performance of lactating dairy cattle. In addition to exploring responses to various mixtures of $NaHCO_3$, NaCl and KCl, a final objective of this study was to examine how CAD was related to response differences.

Calculated CAD values are in Table 4-2 and ranged from +25 to +40. This range of CAD is relatively narrow but is typical of values found in practical rations. For reference, converting NRC (1989) recommendations for Na, K and Cl to a value of CAD results in approximately +25 CAD.

Regression equations were developed by fitting dependent variables to models containing linear and quadratic CAD terms and then sequentially removing terms (from highest to lowest order) that did not contribute to the significance of the regression ($P > .1$). Response to CAD in this range was highly variable. There were only two dependant variables with significant regressions. Milk Cl ($P < .10$; Figure 4-13) responded quadratically and MLMg ($P<.05$; Figure 4-14) responded linearly to increasing CAD. West et al. (1990) compared lactation diets with +2.5, +15, +27.5 and +40 CAD. From figures presented in their report it appeared that dependent variables also were unresponsive in the range of +27.5 to +40 CAD. The findings in this study also are supported by Coppock et al. (1982a), Coppock et al. (1982b), Sanchez et al. (1990a; chapter 3 in this dissertation), and Sanchez et al. (1991; chapter 5 in this dissertation) who observed little response to CAD in the range of +20 to +50. Tucker et al. (1990) compared diets with similar CAD (+32) but different concentrations of NaCl and KCl. This was nearly identical to the NaCl vs. KCl contrast made in the present study. Tucker et al. (1990) also reported slight differences between NaCl and KCl. Plasma K, plasma Ca, urine K, and DMI were lower whereas urine Na and milk fat percentage were higher with NaCl compared with KCl. Overall it was concluded that CAD was a more important determinant of dietary impact on systemic acid-base status than individual concentrations of Na, K and Cl. Most of the effects of CAD on acid-base status and productive functions appear to occur at less than +20 or greater than +50 CAD (Tucker et al., 1988a; West et al., 1990; West et al., 1991; Sanchez et al., 1990a; Sanchez et al., 1991). In the present study, homeostatic

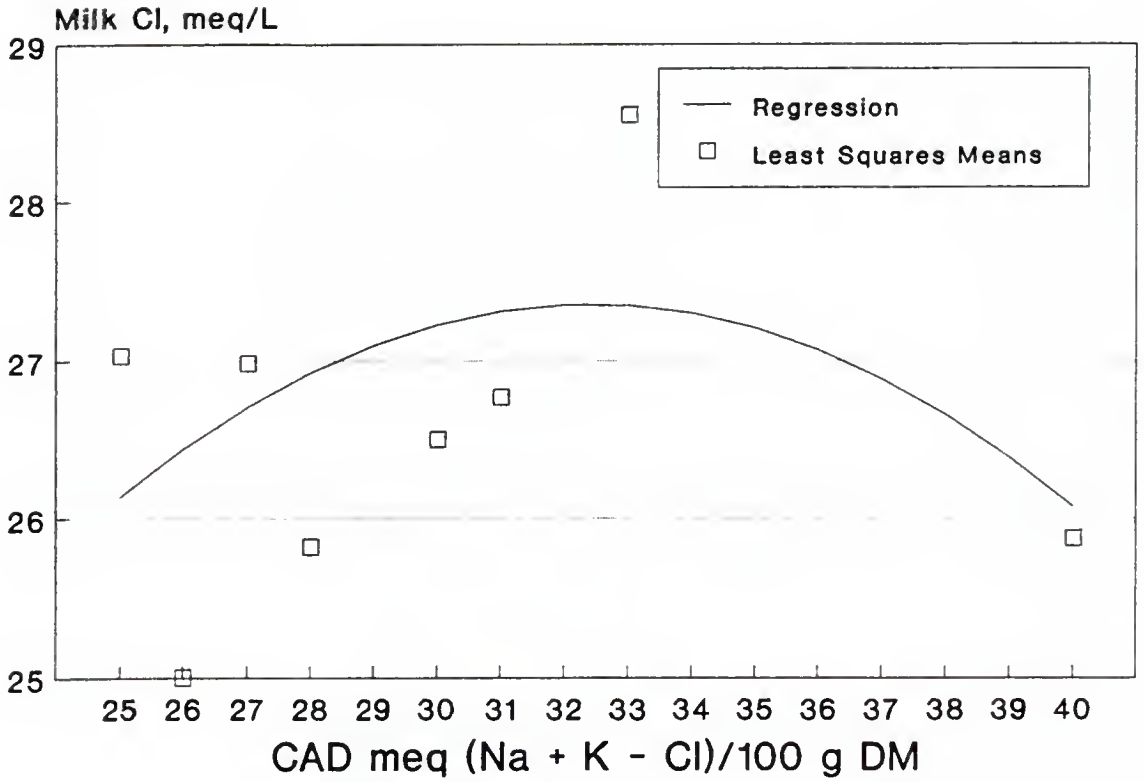

Figure 4-13. Regression of milk Cl (MLCl, meq/L) response to cation anion difference (CAD). Control diet included in regression. Regression for MLCl with SE of coefficients in parentheses: MLCl = 4.04 + 1.44 x CAD (.85) - .0222 x CAD^2 $(.013)^+$. SEM = .88. $^+P < .10$.

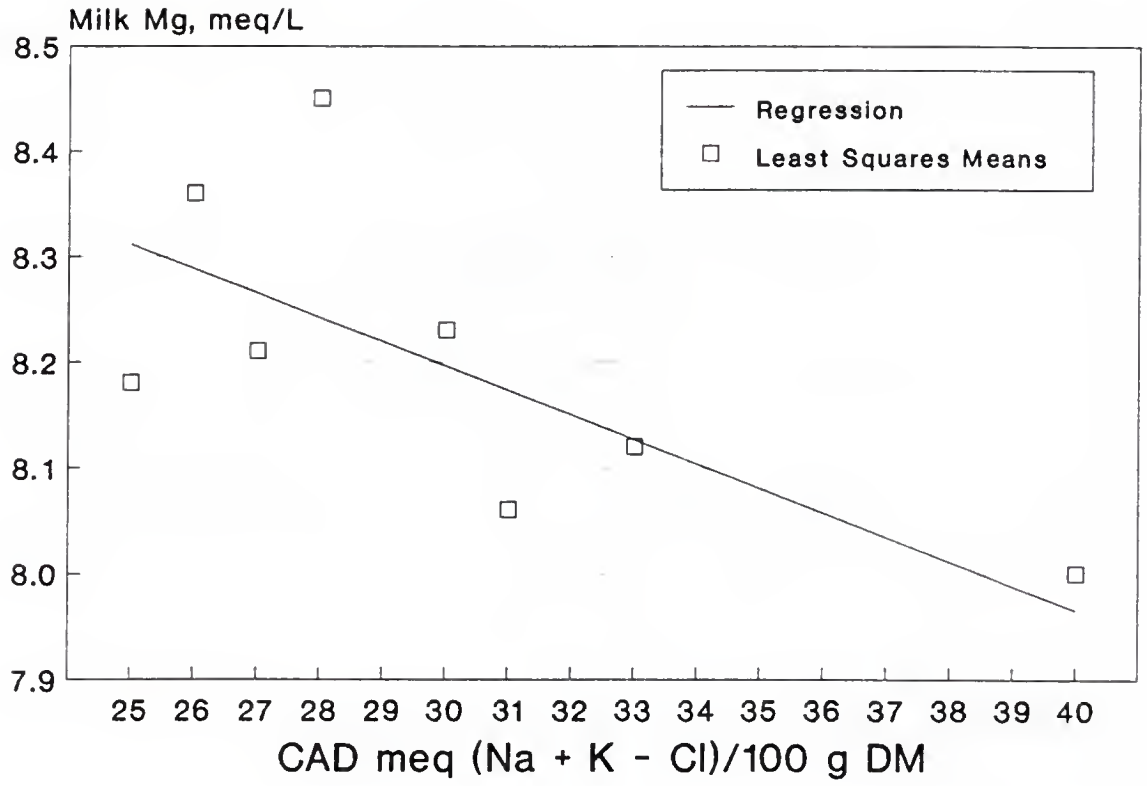

Figure 4-14. Regression of milk Mg (MLMg, meq/L) response to cation anion difference (CAD). Control diet included in regression. Regression for MLMg with SE of coefficients in parentheses: MLMg = 8.89 - .023 x CAD (.01)*. SEM = .14. *P < .05.

mechanisms appear to have been adequate to maintain acid-base status and lactational performance between +25 and +40 CAD. This was reflected by the lack of response to feeding different mixtures of $NaHCO_3$, NaCl and KCl.

Conclusions

Mixtures of $NaHCO_3$, NaCl and KCl fed to lactating dairy cows led to small changes in mineral metabolism and acid-base status but, with the exception of milk protein concentration and body weight gain, did not affect responses associated with lactational performance. There were no apparent benefits from adding $NaHCO_3$, NaCl and/or KCl to a diet that exceeded NRC (1989) recommendations for Na, K and Cl and contained +25 to +40 CAD.

CHAPTER 5
INFLUENCE OF DIETARY MACROMINERAL INTERRELATIONSHIPS AND CATION-ANION DIFFERENCE ON LACTATIONAL PERFORMANCE: USING A LARGE DATA SET AND EMPIRICAL MODELS TO IDENTIFY AND QUANTIFY EFFECTS

Introduction

Dietary macrominerals are necessary for proper health and productive performance of lactating dairy cattle. As a class of nutrients, they have been the subject of substantial investigative efforts (NRC, 1989), and considerable information exists on the individual effects of each macromineral. Information on macromineral interrelationships in diets for lactating dairy cattle is sparse but several two-factor (Mallonee et al., 1982; Schneider et al., 1986; Sanchez et al., 1990a; chapter 3 in this dissertation) and three-factor (Tucker et al., 1988a; West et al., 1990 and 1991) interactions have been identified.

Interrelationships among the monovalent macrominerals (Na, K and Cl) are particularly influential. Mongin (1980) reviewed these interrelationships in nonruminants and suggested that net acid intake could be extrapolated from the difference between the positive (Na and K) and negative (Cl) monovalent macromineral ions. Tucker et al. (1988a) evaluated this concept in lactating dairy cattle with diets formulated to provide varying sums of this cation-anion difference (CAD) expression, calculated as meq (Na + K - Cl)/100 g diet DM. Dry matter intake increased 11% and milk yield (MY) increased 9% with +20 compared

with -10 CAD, independent of the effects of the individual minerals (Na, K or Cl) used to alter CAD.

No experiments have considered all possible interrelationships among macrominerals. The reason is obvious. There are seven macrominerals and to conduct a seven-factor macromineral study with multiple concentrations of each mineral would require an infeasible number of dietary treatments. Alternatives to the classic full factorial experimental designs are available but contain the same inherent problems of requiring many more dietary treatments than can be managed adequately.

Combining data from many studies into one analysis provides a feasible alternative to investigate dietary macromineral interrelationships (Mann, 1990). This approach has been used to quantify the influence of DM, NE_L, CF, NDF, CP, metabolizable protein, and bypass protein intake on lactational performance of lactating dairy cattle (Brown et al., 1977; Briceno et al., 1987; Briceno et al., 1988) and to quantify nutritional risk factors associated with milk fever (Oetzel, 1991). Brown et al. (1980) suggested that summarizing records already accumulated can yield significant results in applied research. Combining data from macromineral studies to identify and quantify effects of macromineral interrelationships and CAD on lactational performance of dairy cattle apparently has not been done, but the need is apparent. Identifying and quantifying these interrelationships will help in designing future research that can evaluate selected interrelationships more adequately. Ultimately they can be incorporated into prediction and nutrient requirement models used by the dairy

industry. Objective of this analysis was to identify and quantify effects of dietary macromineral interrelationships and CAD on lactational performance of dairy cattle.

Materials and Methods

Data Base

Data originated from ten macromineral-nutrition studies conducted at the University of Florida during the 1980's. Each of the studies utilized partially balanced or balanced incomplete block designs, blocked by cow and experimental period. Most cattle were mature and in midlactation. Periods lasted between 28 to 35 d and studies included 3 to 5 periods each. Cows were housed as groups in freestall barns equipped with individual feeding systems (American Calan, Inc., Northwood, NH) and fed and milked twice daily.

Dry matter intake (DMI) and milk yield (MY) data were collected during the last 2 wk of each period in all but one study. In Morse et al. (1989) data were collected during the last wk. The data base represented over 14,000 daily feed intake and MY measurements and over 4000 separate milk composition measurements. This yielded 1022 cow-period means (937 Holstein and 85 Jersey). Cow-period means for DMI and MY used in the data base were averages of daily measures. Milk samples usually were taken during the last 6 milkings (depending on specific study protocol). Cow-period means for 4% fat-corrected milk (4% FCM) yield and milk composition were averages of individual milkings (weighted according to corresponding milk weight). Because milk samples were not taken in all studies, 4% FCM yield, and milk composition

variables were represented by only 757 cow-period means (all from Holsteins). Data from a total of 326 cows were included in the data base. Included were data from two seasons (winter and summer), two forage types (corn silage and cottonseed hulls), and two breeds (Holstein and Jersey).

Table 5-1 provides additional information about studies included in the data base. Means, SEM, minimum and maximum macromineral concentrations from combined studies are presented in Table 5-2. Corresponding NRC (1989) recommendations and CAD values are included for comparison.

Statistical Models

Cow-period means from all experiments were combined and analyzed by least squares ANOVA using general linear models procedures of SAS (1985). Full least squares statistical models included both discrete and continuous independent variables. Sources of variation from discrete effects included season, study nested within season, cows nested within study and season, period, and forage type. Breed was not included in least squares models as a discrete effect; including cow nested within study and season accounted for variation due to breed. Continuous independent variables were linear, quadratic, and two-way macromineral interaction terms in one analysis (macromineral models); and linear, quadratic and cubic polynomial CAD terms in another (CAD models). Sulfur, which was not investigated in any of the original studies, was not included as a source of variation in statistical

TABLE 5-1. Type of study, cow-period observations, season, forage type, and reference of studies included in data base.

Study	Type of Study	Cow-period observations	Season	Forage Type	Reference
1	Factorial Na x K	64 Holstein 44 Jersey	Winter	Cottonseed hulls	Mallonee et al. (1982)
2	Factorial Na x K	49 Holstein 17 Jersey[1]	Summer	Cottonseed hulls	Schneider et al. (1984b
3	K	30 Holstein 24 Jersey	Summer	Cottonseed hulls	Mallonee et al. (1985)
4	Factorial Na x K	71 Holstein	Summer	Corn silage	Schneider et al. (1986)
5	Na x Forage type	102 Holstein	Summer	Corn silage Cottonseed hulls	Beede et al. (1987)
6	Factorial Na x K x Mg	138 Holstein	Winter	Corn silage	O'Connor et al. (1988)
7	Factorial Ca x P	72 Holstein	Winter	Corn silage[2]	Morse et al. (1989)
8	Mg	112 Holstein	Winter	Corn silage[3]	Lough et al. (1990)
9	Factorial Na x K x Cl	192 Holstein	Winter	Corn silage[4]	Sanchez et al. (1990a)
10	Mixtures Na x K x Cl	107 Holstein	Summer	Corn silage[5]	Sanchez et al. (1990b)

[1]Jersey data from this study were not reported in cited reference but were included in data base.

[2]Contained 49% corn silage and 15% cottonseed hulls, DM basis; classified as corn silage-forage type.

[3]Contained 41% corn silage and 4% cottonseed hulls, DM basis; classified as corn silage-forage type.

[4]Contained 40% corn silage and 5.5% cottonseed hulls, DM basis; classified as corn silage-forage type.

[5]Contained 40% corn silage and 13% whole cottonseeds, DM basis; classified as corn silage-forage type.

TABLE 5-2. Concentrations of dietary macrominerals and cation-anion difference: mean, SEM and ranges in data base. Recommended macromineral concentrations shown for comparison.[1]

Macromineral[1]	Mean	SEM	Range	NRC[2]
Na, %	.49	.007	.11 to 1.20	.18
K, %	1.30	.009	.66 to 1.96	.90
Cl, %	.78	.011	.15 to 1.45	.25
Ca, %	.83	.004	.50 to 1.08	.58
P, %	.45	.002	.33 to .65	.37
Mg, %	.33	.003	.21 to .62	.20
CAD, meq (Na + K - Cl)/ 100 g diet DM	32.4	...	5.8 to 61.2	...

[1]No studies involving S were in data base; S which averaged .2% was not included in models.

[2]NRC (1989) recommended nutrient concentrations for 500 kg cow producing 25 kg of milk/d.

models. Full models were reduced by removing nonsignificant terms (P > .1) via partial sums of squares tests. If an interaction or quadratic term in the polynomial was significant, the associated linear terms remained in the model regardless of P value.

Results and Discussion

Tables 5-1 and 5-2, as indicated previously, provide a description of data used in this analysis. Nearly all studies evaluated more than one macromineral. When combined into one data base, concentrations of macrominerals ranged from below recommendations (NRC, 1989) to concentrations considerably above recommendations. As is typical in many commercial feeding programs, mean macromineral concentrations were higher than NRC (1989) recommendations. Sulfur concentration averaged .2% of diet DM. Cation-anion difference ranged from +5.8 to +61.2 meq/100 g. Overall, DMI, MY, 4% FCM yield, milk fat percentage and milk protein percentage averaged 21.8 kg/d, 22.8 kg/d, 21.5 kg/d, 3.50%, and 3.19% respectively. Over 90% of data were from Holstein records. Jersey data were from the first three studies and consisted only of DMI and MY (Table 5-1). Milk from Jersey's was not sampled for composition. Jersey data were not reported in the cited reference for experiment 2, but that data were included in this analysis.

Studies conducted during summer had fewer cow-period observations than studies conducted during winter (400 vs. 622). Effect of season was highly significant (Table 5-3 and 5-5). Parameter estimates (averaged across macromineral and CAD models) revealed that DMI was 2.96 kg/d greater, MY .03 kg/d less, 4% FCM yield 1.05 kg/d greater,

milk fat .396 percentage units greater, and milk protein .108 percentage units greater during the winter season.

Corn silage-based diets were fed more often than cottonseed hull-based diets (746 vs. 279 cow-period observations). In study 6 (Beede et al. 1987), 54 cow-period observations were from corn silage based-diets, whereas 48 were from cottonseed hull-based diets. Depending on treatment specifications within each experiment, small amounts of cotton products were included in several of the corn silage-based diets but these diets still were classified as corn silage-forage type for purposes of this analysis. Variation due to nutrient and energy concentrations were accounted for by the variation associated with different studies (i.e., with the exception of macrominerals, nutrient and energy concentrations were fixed within studies). Due to the known interaction between forage type and mineral supplementation (i.e., from dietary buffers) on production performance (Erdman, 1988; Staples and Lough, 1989), the influence of forage type was removed from specific effects of macrominerals by including it as a source of variation. Forage type had an effect on DMI ($P \leq .001$) and 4% FCM yield ($P \leq .05$) in macromineral models (Table 5-3) and on DMI ($P \leq .001$), MY ($P \leq .1$), 4% FCM yield ($P \leq .01$) in CAD models (Table 5-5). Parameter estimates revealed that DMI was 2.86 kg/d greater, MY 1.05 kg/d greater, and 4% FCM yield 1.56 kg/d greater with corn silage- compared to cottonseed hull-based diets. The reason for the greater DMI with corn-silage compared to cottonseed hull-based diets may actually have been due to the Jersey's that were included in the first three studies rather than a specific forage effect.

Macromineral Models

Least squares ANOVA for macromineral models is presented in Table 5-3. Reduced models with corresponding standard errors of coefficient estimates are presented in Table 5-4. The three-dimensional response surfaces in Figures 5-1 through 5-4 illustrate important linear, curvilinear and two-way interaction effects of dietary macrominerals.

There were several macromineral interrelationships that had consistent effects across different response variables. A Na x K interaction influenced DMI, 4% FCM yield and milk fat percentage (Figure 5-1). Response surfaces signified a consistent sparing of one cation for the other on all responses that included a Na x K interaction term. If dietary concentrations of either Na or K were high, DMI, 4% FCM yield and milk fat percentage were greatest when the concentration of the other cation was relatively low. Fontenot et al. (1960) reported that additional dietary Na depressed K absorption in lambs. Increasing dietary K intake in sheep resulted in an increase in fecal Na (Suttle and Field, 1967). Scott (1970) found that high dietary K impaired intestinal absorption of Na. Campbell and Roberts (1965) reported that apparent intestinal absorption of Na in heifers was impaired by high concentration of dietary K but lower concentrations of K increased urinary excretion of Na. Scott (1967) observed that an increase in the ruminal fluid concentration of one of these ions is accompanied by a reciprocal decrease in the other, resulting in an almost constant meq concentration of the sum of Na plus K. In contrast, Erdman et al. (1980) did not detect an improvement in lactational performance with additional Na (.52 vs. .31%) with either low (.42%) or adequate (.84%) K

Table 5-3. Least squares analysis of variance for dry matter intake (DMI), milk yield (MY), 4% fat-corrected milk (4% FCM) yield and milk composition from macromineral models.[1]

	DMI (kg/d)		MY (kg/d)		4% FCM Yield (kg/d)		Milk Fat (%)		Milk Protein (%)	
Source	df	MS	df	MS	df	MS	df	MS	df	MS
Season	1	447.91***	1	2253.13***	1	479.06***	1	1.019**	1	2.273***
Study (season)	8	38.43***	8	630.96***	6	205.28***	6	10.112***	6	4.462***
Cow (study season)	320	41.14***	320	68.81***	253	52.77***	253	115.53***	253	.173***
Period	4	72.72***	4	809.17***	4	562.57**	4	13.378***	4	1.898***
Forage type	1	120.82***	...	...	1	22.04*	...	...	...	...
Na	1	3.02	1	27.96*	1	4.35	1	.342†	1	.442**
K	1	.00	...	...	1	22.02*	1	.907**	1	.757***
Cl	1	11.97†	...	...	1	16.92*	1	.329†	1	.383**
Ca	1	21.95*	1	137.41***	1	2.71	1	.740**	1	.123†
P	1	9.28	...	...	1	25.34*	1	.320†	1	.032
Mg	1	36.29*	1	24.95*	1	18.45*	1	.286†	1	.132†
Na x Na	1	33.38**	1	86.46**	...	...	...	...	1	.373**
Mg x Mg	1	38.24**	1	24.80*	1	18.13*	...	...	...	...
P x P	...	...	...	...	1	29.52**	...	...	...	...
Na x K	1	15.06*	...	...	1	14.56†	1	.278†	...	...
Na x Ca	1	14.49*	1	106.97***	1	29.90**	...	...	...	...
Na x P	1	14.75†	...	...	...	...	...	...	1	.218*
K x Cl	1	14.77*	...	...	1	20.06*	...	...	...	...
K x Ca	...	...	...	...	1	20.49*	1	.892**	1	.813***
K x P	...	...	...	...	1	18.60*	1	.361†	...	...
Cl x Ca	...	...	...	...	...	...	...	...	1	.385**
Ca x P	1	18.62*	...	...	...	...	...	...	...	...
P x Mg	...	...	...	...	...	...	...	...	1	.138†
Residual	674	3.88	682	5.53	479	4.48	484	.105	482	.048

[1]Terms not shown were nonsignificant ($P > .1$).

***$P \leq .001$, **$P \leq .01$, *$P \leq .05$, †$P \leq .1$.

TABLE 5-4. Regression coefficients and standard errors of coefficient estimates from reduced macromineral models for dry matter intake (DMI), milk yield (MY) 4% fat-corrected milk (4% FCM) yield, milk fat percentage (MF) and milk protein percentage (MP).[1]

DMI = 28.91 (8.31) + 7.15 Na (8.1) +.03 K (.85) - 2.69 Cl (1.53)† - 19.82 Ca (8.33)* - 23.95 P (15.48) + 26.11 Mg (8.54)** - 4.78 Na x Na (1.63)** - 32.61 Mg x Mg (10.38)** -2.98 Na x K (1.51)* + 10.76 Na x Ca (5.57)* - 14.52 Na x P (7.45)* + 2.16 K x Cl (1.10)* + 38.08 Ca x P (17.38)*.

MY = 28.86 (3.39) - 10.44 Na (4.65)* - 14.45 Ca (2.90)** + 21.48 Mg (10.12)* - 6.97 Na x Na (1.76)** - 26.11 Mg x Mg (12.33)* + 22.37 Na x Ca (5.09)**.

4% FCM = 30.41 (12.77)- 5.60 Na (5.69) + 22.70 K (10.24)* - 4.83 Cl (2.49)* + 8.48 Ca (10.90) - 109.11 P (45.89)* + 23.20 Mg (11.43)* - 29.32 Mg x Mg (14.58)* + 143.24 P x P (55.82)** - 4.14 Na x K (2.30)† + 15.49 Na x Ca (6.0)** + 3.83 K x Cl (1.81)* - 21.36 K x Ca (9.99)* - 13.39 K x P (6.57)*.

MF = -.45 (1.511) + .850 Na (.471) + 3.96 K (1.350)** - .132 Cl (.075)† + 3.478 Ca (1.310)** + 2.065 P (1.829)† - .435 Mg (.263) † -.544 Na x K (.335)† - 3.561 K x Ca (1.222)** - 1.712 K x P (.924)†.

MP = 1.67 (.920) - 1.803 Na (.593)** + 2.172 K (.545)** - 1.936 Cl (.684)** + 1.306 Ca (.815)† + .866 P (1.065) + 2.100 Mg (1.265)† + .905 Na x Na (.323)** + 1.960 Na x P (.918)* - 2.638 K x Ca (.639)** + 2.312 Cl x Ca (.815)** -4.00 P x Mg (2.352)†.

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[1]Standard errors of coefficients are in parentheses. Terms not shown were nonsignificant ($P > .1$). Pooled standard errors of the mean = .14 kg/d, .19 kg/d, .20 kg/d, .088 % and .046 % for DMI, MY, 4% FCM yield, MF and MP, respectively.

** $P \leq .01$.; * $P \leq .05$; † $P \leq .1$

Table 5-5. Least squares analysis of variance for dry matter intake (DMI), milk yield (MY), and 4% fat-corrected milk (4% FCM) yield for cation-anion difference (CAD) models.[1]

Source	DMI (kg/d)		MY (kg/d)		4% FCM Yield (kg/d)	
	df	MS	df	MS	df	MS
Season	1	713.66***	1	1500.62***	1	1787.35***
Study (season)	8	112.46***	8	592.13***	6	728.44***
Cow (study season)	320	42.49***	320	7.11***	253	53.66***
Period	4	70.08***	4	799.95***	4	560.93***
Forage type	1	130.99***	1	16.17†	1	43.66**
CAD	1	33.55**	1	68.42***	1	47.38***
CAD × CAD	1	26.94**	1	57.37***	1	41.50**
Residual	685	4.085	685	5.82	490	4.62

[1]Terms not shown were nonsignificant ($P > .1$); CAD [meq (Na + K - Cl)/100 g of diet DM] did not influence milk fat or protein percentage ($P > .1$).

***$P \leq .001$, **$P \leq .01$, †$P \leq .1$.

A

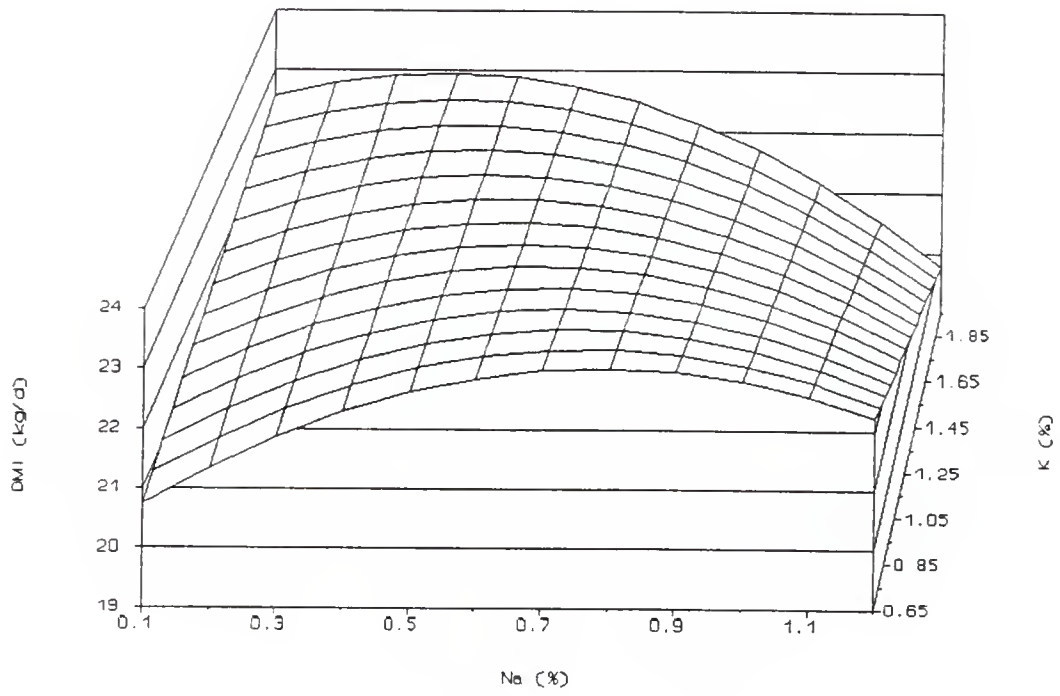


Figure 5-1. Response surfaces for dry matter intake (DMI) (A), 4% FCM yield (B) and milk fat (C) plotted against dietary Na and K with all other macrominerals at their mean concentrations. Pooled standard error of means = .14 kg/d, .20 kg/d and .088% for DMI, 4% FCM yield and milk fat, respectively.

B

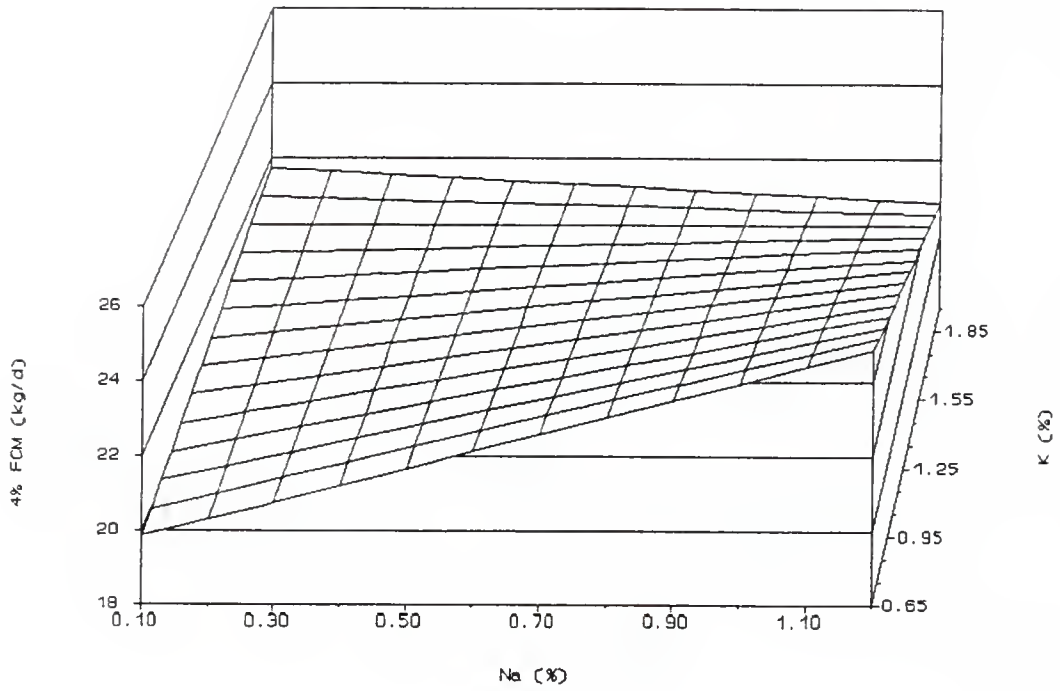


Figure 5-1--continued. Response surfaces for dry matter intake (DMI) (A), 4% FCM yield (B) and milk fat (C) plotted against dietary Na and K with all other macrominerals at their mean concentrations. Pooled standard error of means = .14 kg/d, .20 kg/d and .088% for DMI, 4% FCM yield and milk fat, respectively.

C

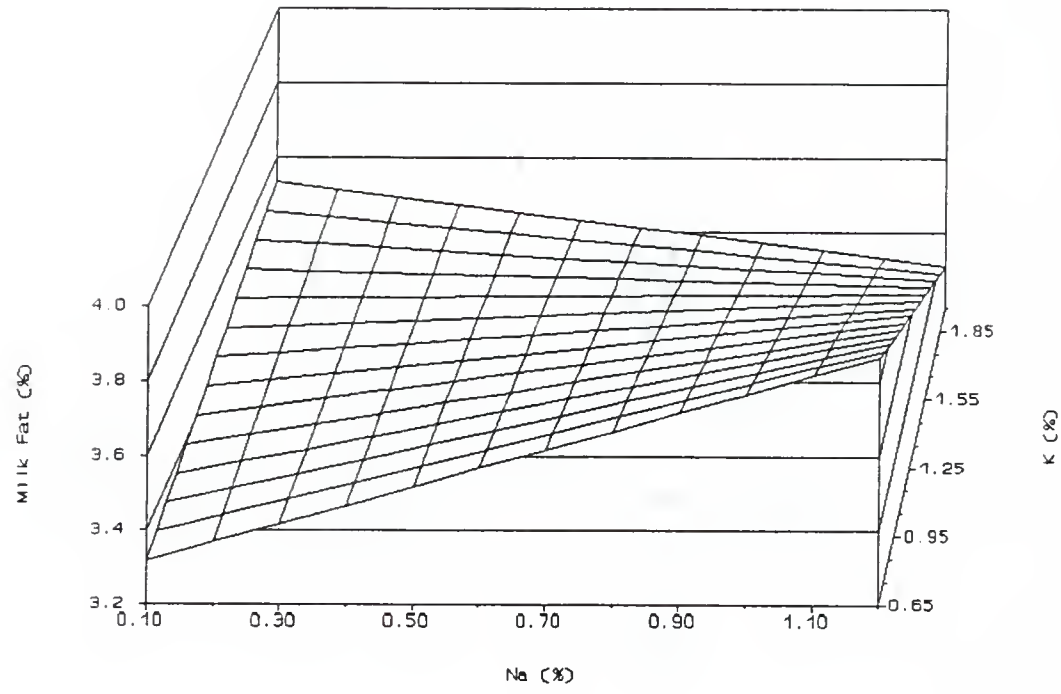


Figure 5-1--continued. Response surfaces for dry matter intake (DMI) (A), 4% FCM yield (B) and milk fat (C) plotted against dietary Na and K with all other macrominerals at their mean concentrations. Pooled standard error of means = .14 kg/d, .20 kg/d and .088% for DMI, 4% FCM yield and milk fat, respectively.

A

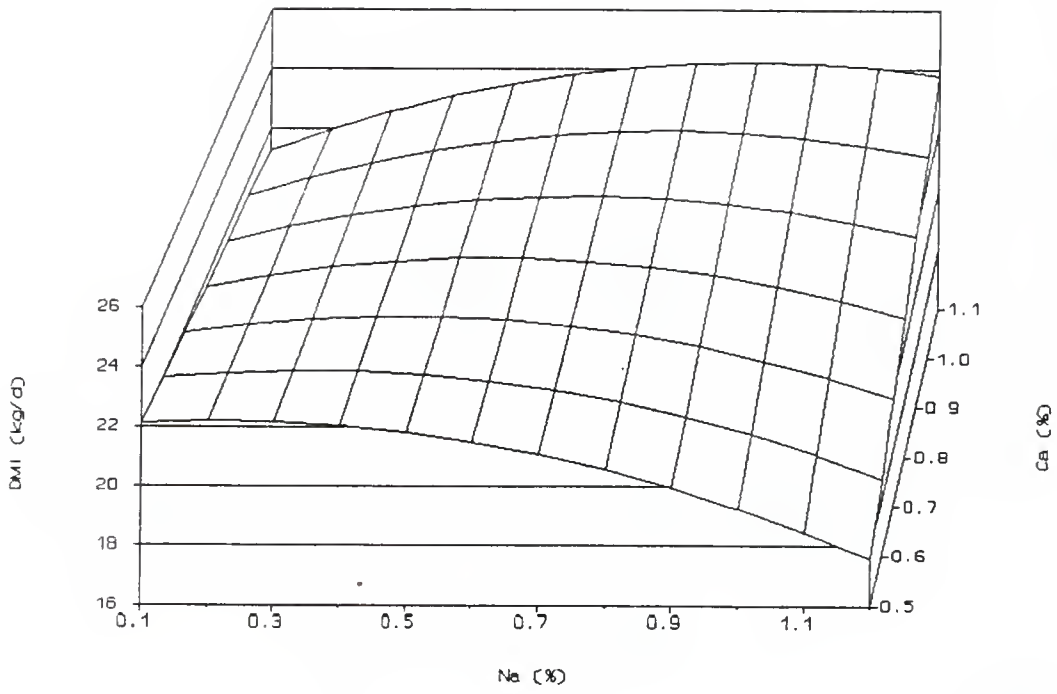


Figure 5-2. Response surfaces for dry matter intake (DMI) (A), milk yield (B) and 4% FCM yield (C) plotted against dietary Na and Ca with all other macrominerals at their mean concentrations. Pooled standard error of means = .14 kg/d, .19 kg/d and .20 kg/d for DMI, milk yield and 4% FCM yield, respectively.

B

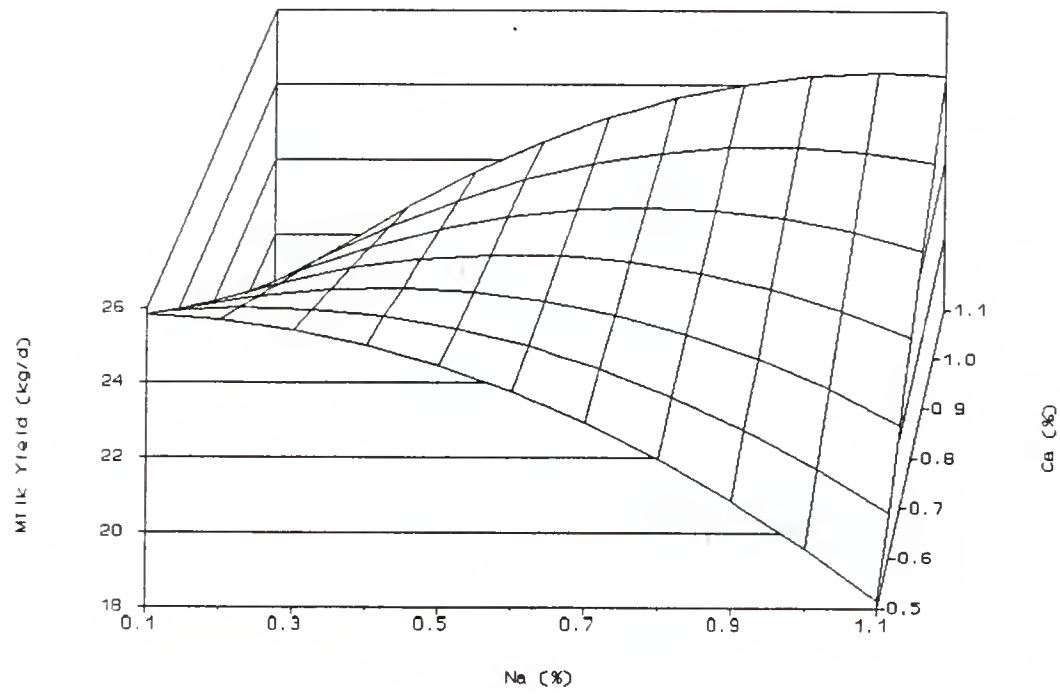


Figure 5-2--continued. Response surfaces for dry matter intake (DMI) (A), milk yield (B) and 4% FCM yield (C) plotted against dietary Na and Ca with all other macrominerals at their mean concentrations. Pooled standard error of means = .14 kg/d, .19 kg/d and .20 kg/d for DMI, milk yield and 4% FCM yield, respectively.

C

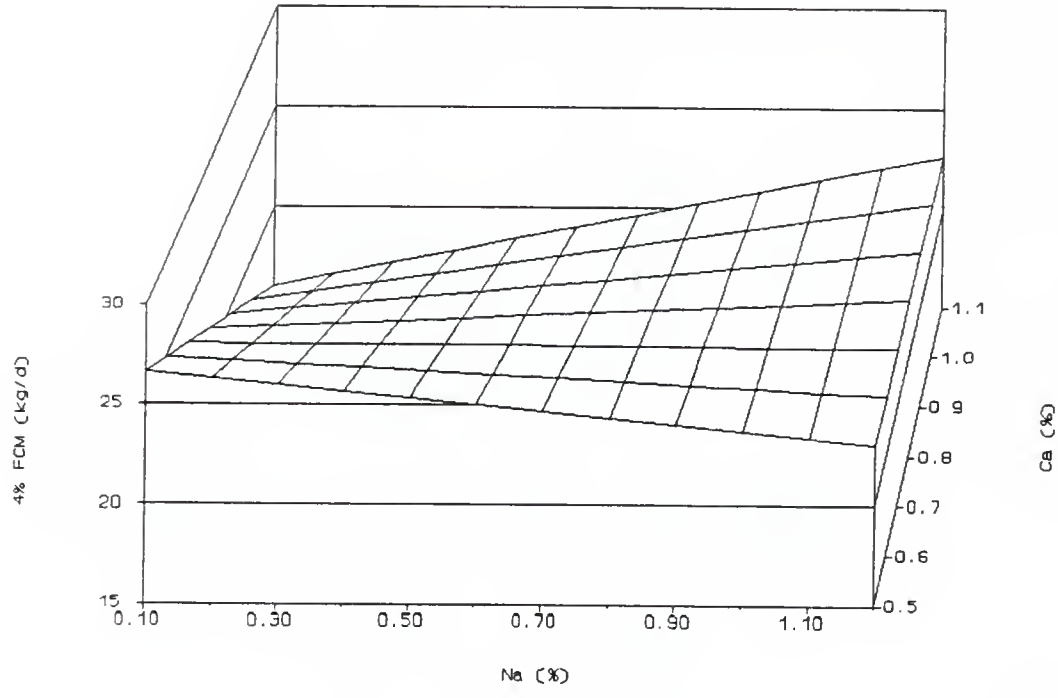


Figure 5-2--continued. Response surfaces for dry matter intake (DMI) (A), milk yield (B) and 4% FCM yield (C) plotted against dietary Na and Ca with all other macrominerals at their mean concentrations. Pooled standard error of means = .14 kg/d, .19 kg/d and .20 kg/d for DMI, milk yield and 4% FCM yield, respectively.

A

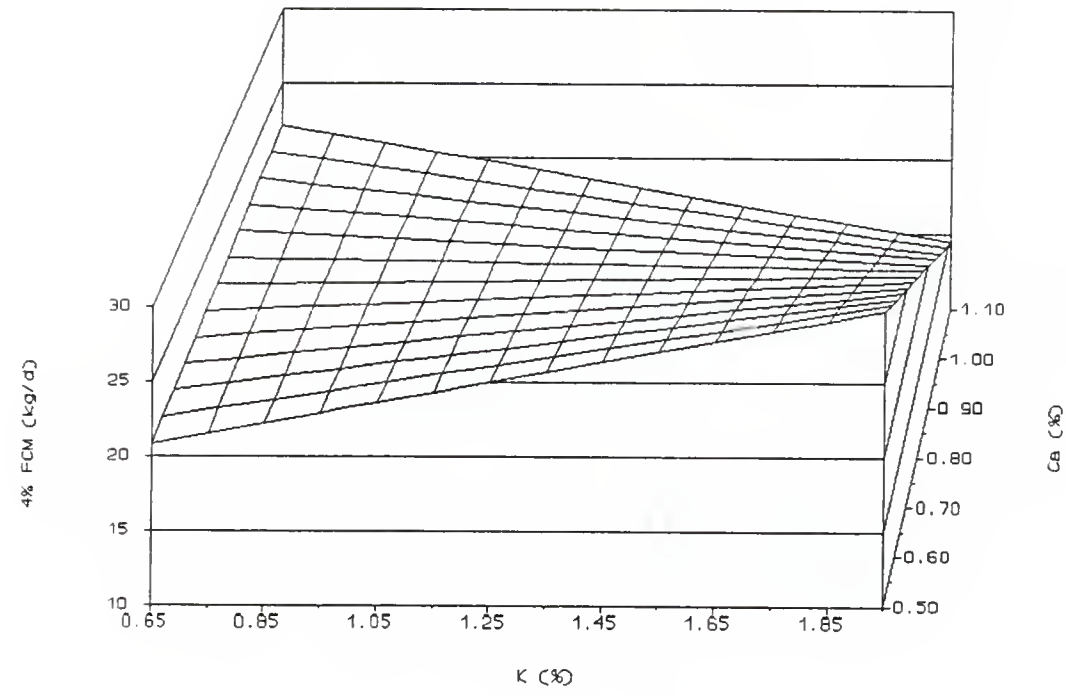


Figure 5-3. Response surfaces for 4% FCM yield (A), milk fat (B) and milk protein (C) plotted against dietary K and Ca with all other minerals at their mean concentrations. Pooled standard error of means = .20 kg/d, .088 % and .046% kg/d for 4% FCM yield, milk fat and milk protein, respectively.

B

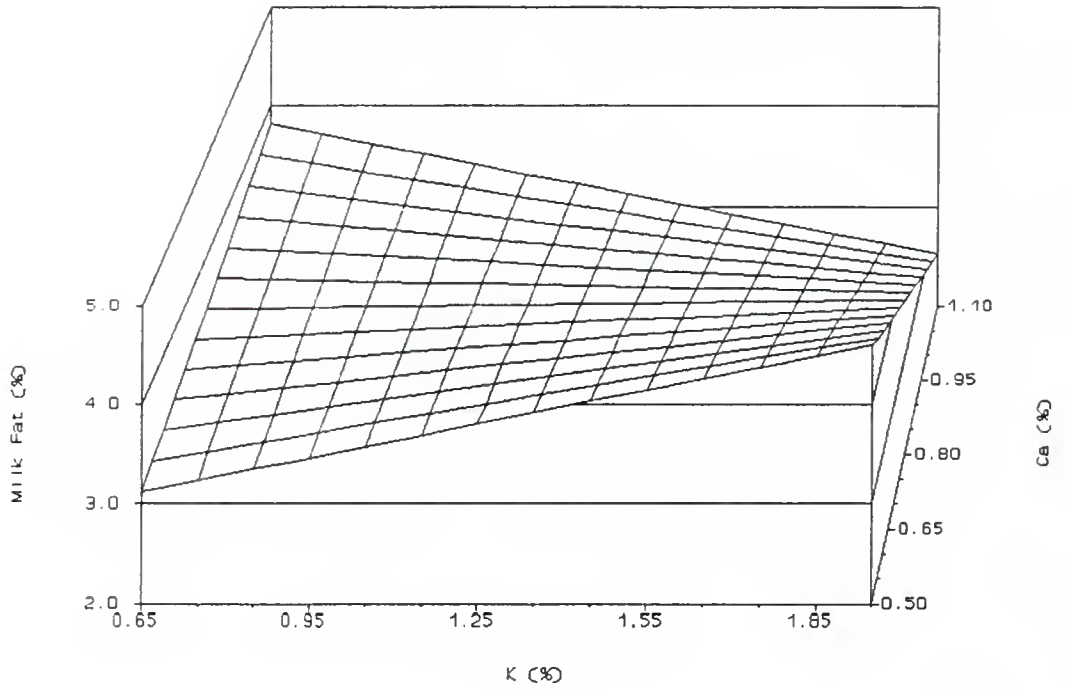


Figure 5-3--continued. Response surfaces for 4% FCM yield (A), milk fat (B) and milk protein (C) plotted against dietary K and Ca with all other minerals at their mean concentrations. Pooled standard error of means = .20 kg/d, .088 % and .046% kg/d for 4% FCM yield, milk fat and milk protein, respectively.

C

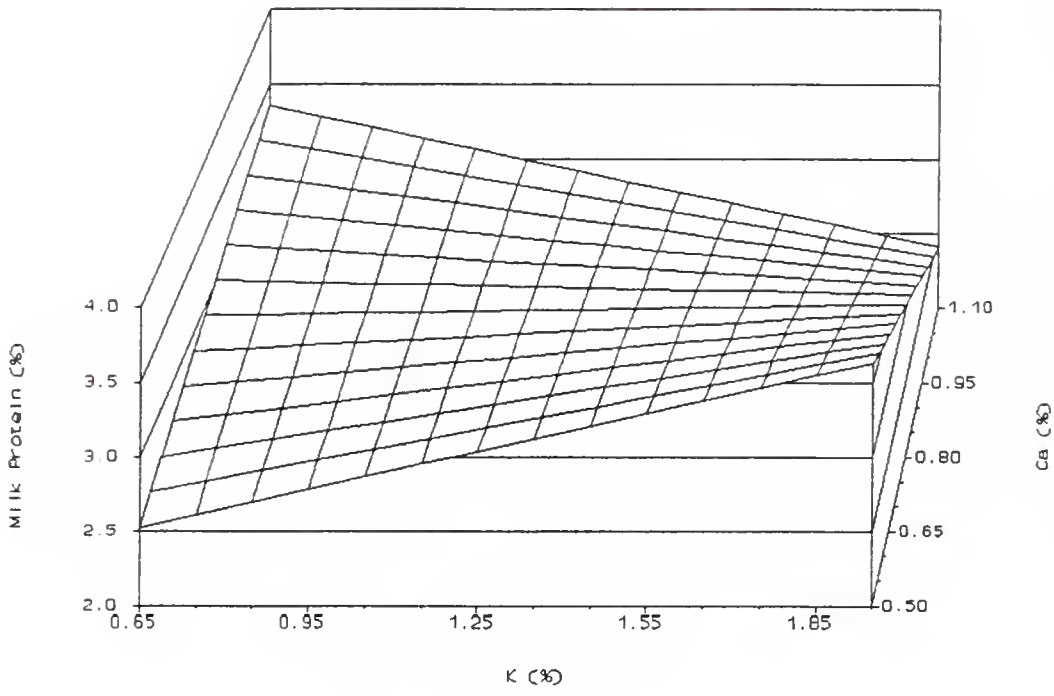


Figure 5-3--continued. Response surfaces for 4% FCM yield (A), milk fat (B) and milk protein (C) plotted against dietary K and Ca with all other minerals at their mean concentrations. Pooled standard error of means = .20 kg/d, .088 % and .046% kg/d for 4% FCM yield, milk fat and milk protein, respectively.

A

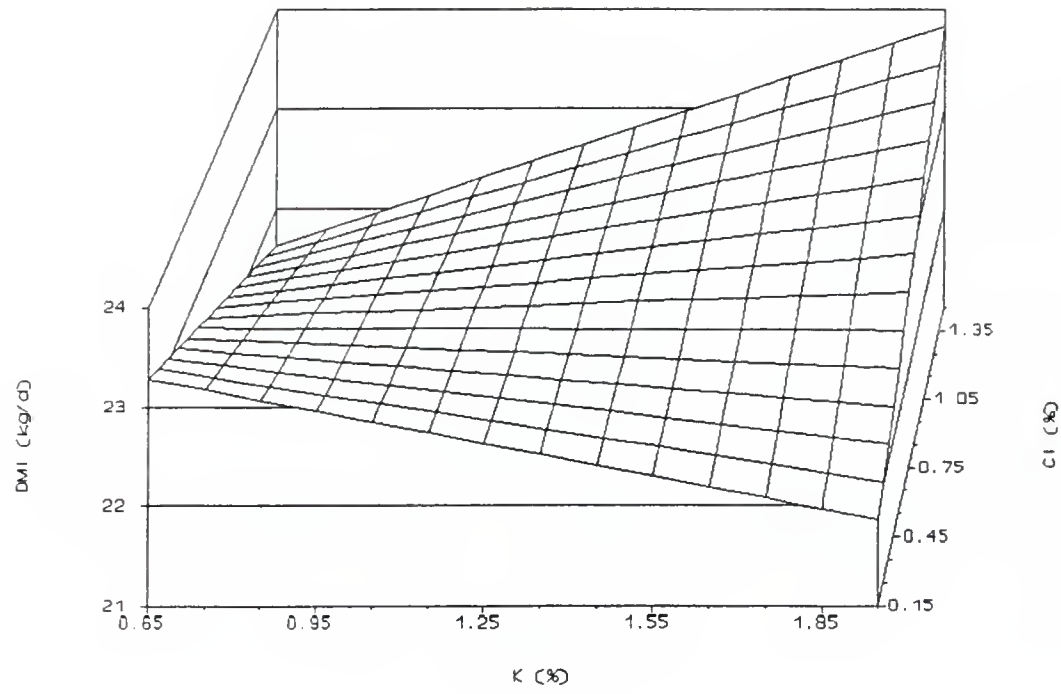


Figure 5-4. Response surfaces for dry matter intake (DMI) (A) and 4% fat-corrected milk (4% FCM) yield (B) plotted against dietary K and Cl with all other macrominerals at their mean concentrations. Pooled standard error of means = .14 kg/d and .20 kg/d for DMI and 4% FCM yield, respectively.

B

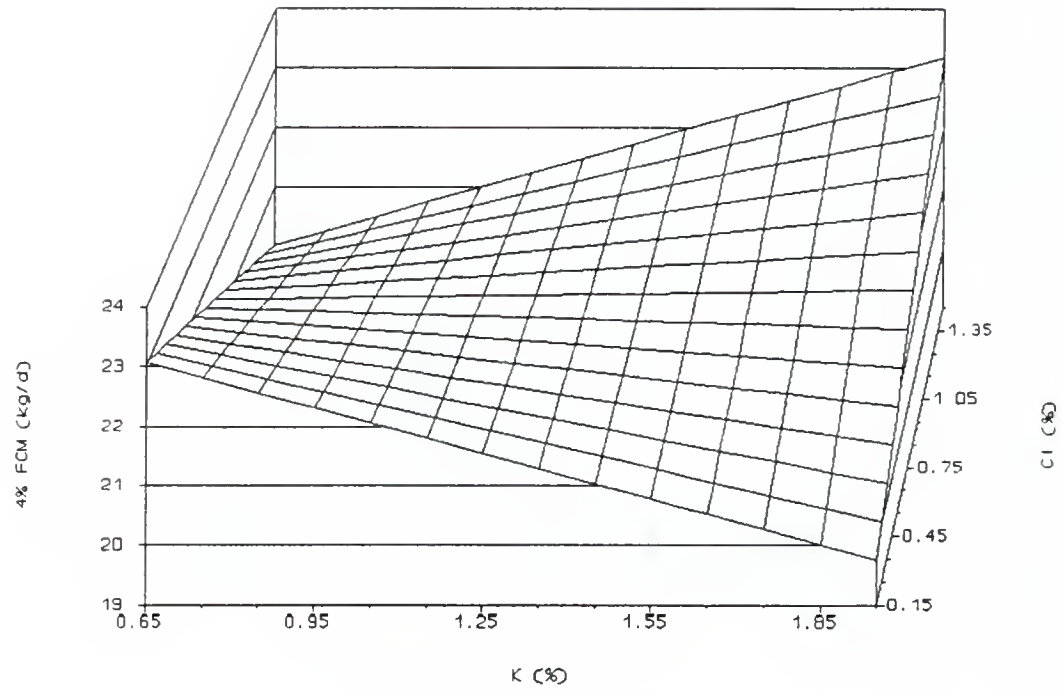


Figure 5-4--continued. Response surfaces for dry matter intake (DMI) (A) and 4% fat-corrected milk (4% FCM) yield (B) plotted against dietary K and Cl with all other macrominerals at their mean concentrations. Pooled standard error of means = .14 kg/d and .20 kg/d for DMI and 4% FCM yield, respectively.

but a sparing effect of dietary Na on K was suggested by serum K response. Chloride was not equalized across diets in that study which may explain the lack of MY effects. Martens and Blume (1987) observed in vivo that Na and Cl absorption in sheep was coupled by a dual exchange mechanism of Na^+/H^+ and Cl^-/HCO_3^- and was related to K concentration in the rumen. An alteration in the relative amounts of dietary Na and K thus could be expected to affect acid-base status of the gastrointestinal tract. Acid-base disturbances might explain the interaction and sparing effects of Na and K on milk fat percentage due to the well known dependance of milk fat synthesis on ruminal pH (Erdman, 1988; Staples and Lough, 1989).

An interaction between Na and Ca also influenced three response variables (DMI, MY and 4% FCM yield; Figure 5-2). Response to increased dietary concentrations of Na was small or negative when concentration of Ca was held low. However, if increased dietary Na was accompanied by increased Ca, DMI, MY and 4% FCM yield increased. Apparently an interaction between Na and Ca has not been investigated previously. A possible explanation for the nature of the relationship can be inferred from the findings of Paquay et al. (1968). These researchers summarized numerous balance trials with dry and lactating cattle and examined the relationship between calcium metabolism and other dietary factors. Significant correlations between Na intake and fecal Ca ($r = .449$; $P < .01$), urinary Na and Ca digestibility ($r = -.338$; $P < .01$), and urinary Na and Ca balance were noted. They suggested that factors other than dietary Ca influenced Ca balance. These correlations implied that increasing dietary Na was detrimental to Ca balance (when Ca was not

increased also). This could explain why in the present analysis, increasing dietary Na was most beneficial when accompanied by concomitant increases in dietary Ca.

Four percent FCM yield, milk fat percentage and milk protein percentage were all influenced by dietary K x Ca interaction (Figure 5-3). Response surfaces were remarkably similar. Considering the fact that 4% FCM yield was affected by both Na x K and Na x Ca interactions, it is logical that K x Ca interaction might also influence 4% FCM yield. It also is logical that the nature of the K x Ca interaction was opposite that of the Na x Ca interaction. The nature of the K x Ca interaction indicated that K spared Ca (or vice versa). Increasing dietary K from .65% to 1.95% with dietary Ca at low concentrations, elevated milk fat and protein in milk. But when dietary Ca was high, an increase in dietary K lowered milk fat and protein concentrations. Erdman et al. (1980) did not observe K x Ca interactions on milk production responses but did observe interrelated effects of Ca and K on fecal pH and serum K. In their study, increasing Ca from .5 to 1.03% increased fecal pH to a greater extent with .52% K than with .77 or 1.04% K diets. Increasing fecal pH could have represented an improvement in acid-base homeostasis which could explain why, in the present analysis, milk production was improved with increasing dietary Ca when K was low. Diets from Erdman et al. (1980) were not isochloridic so there may have been masked effects from dietary Cl. It is also possible that the effect ascribed to Ca in Erdman et al. (1980) was due to lower tract buffering of limestone and not due to Ca per se (Erdman, 1988).

All other significant interrelationships affected no more than two response variables. A K x Cl interaction (Figure 5-4) affected DMI and 4% FCM yield. Although not included in the reduced model, K x Cl also tended to affect milk protein percentage (P = .14). To date, interrelationships between K and Cl on milk production have not been reported for lactating dairy cattle. Data of Tucker et al. (1988a) provide evidence for a K x Cl interaction on MY. With a relatively high Cl diet (1.25%), there was a positive linear MY increase with increasing dietary K (from .73 to 1.91% K). Lower concentrations of Cl were not tested in combination with that range of dietary K so this cannot be confirmed conclusively from their report. Golz and Crenshaw (1990) recently reported a dietary K x Cl interaction on growth of weanling pigs. In parallel with the findings in the present analysis, with low concentrations of K, an increase in dietary Cl depressed growth, whereas at high concentrations of K, an increase in Cl improved growth.

A possible explanation of the K x Cl interaction in present study may be derived from the findings of Paquay et al. (1969). Paquay et al. (1969) observed a positive correlation between K and Cl in the urine of cows. They reasoned that because dairy cattle are herbivorous they often consume excess K. Excess K is excreted through the urine. But to maintain electric neutrality of the urine an anion must also be excreted to accompany K cations. Chloride, which is in greatest concentrations in ruminant extracellular fluid (Lunn and McGuirk, 1990), is the anion that most often accompanies K in the urine. Physiological needs for K and Cl may have accounted for the dietary K x Cl interaction on lactational performance.

Interactions between Na and P on DMI and milk protein and interactions between K and P on 4% FCM yield and milk fat percentage also were observed (Table 5-3). The biological significance of these interrelationships could not be interpreted.

One objective of dairy cattle nutrition research is to determine the concentration of a nutrient that yields a maximum production response. It is usually of value to mathematically pinpoint the position of the response maximum, if it exists. Maximum points, calculated with derivatives, equal the point where the response slope is equal to zero. For most macrominerals, no single maximum existed and response surfaces were planar. The numerous interrelationships that existed among these macrominerals may be responsible. In spite of these interrelationships, a specific concentration of Na and Mg maximized DMI, MY and 4% FCM yield. When all other minerals were fixed at their mean concentrations, .59% Na and .40% Mg maximized DMI; .58% Na and .41% Mg maximized MY; and .40% Mg maximized 4% FCM yield. These maximums are readily apparent in response surfaces presented in Figure 5-5.

Cation-Anion Difference Models

Least squares ANOVA for CAD models is presented in Table 5-5. Reduced models with corresponding standard errors of coefficient estimates are presented in Table 5-6. Figure 5-6 illustrates how CAD influenced DMI, MY and 4% FCM yield. Derivatives indicated that DMI, MY and 4% FCM yield were all maximum at +38 CAD. Cation-anion difference did not influence milk composition.

TABLE 5-6. Regression coefficients with standard errors of coefficient estimates from reduced cation-anion difference (CAD) models for dry matter intake (DMI), milk yield (MY), and 4% fat-corrected milk (4% FCM) yield.[1]

DMI	= 20.0211 (1.56) + .11353973 **CAD** (.04) - .00147457 **CAD x CAD** (.0006)**.
MY	= 20.29433 (1.86) + .16214773 **CAD** (.05) - .00215180 **CAD x CAD** (.0007)**.
4% FCM	= 18.34 (1.71) + .15116144 **CAD** (.05) - .00200643 **CAD x CAD** (.0007)**.

[1]Standard errors of coefficients are in parentheses. Terms not shown were nonsignificant ($P > .1$). Linear or quadratic effect of CAD [meq (Na + K - Cl)/100 g of diet DM] was not significant for milk fat or milk protein percentage. Pooled standard errors of the mean = .14 kg/d, .19 kg/d, and .20 kg/d for DMI, MY, and 4% FCM yield, respectively.

** $P \leq .01$.

A

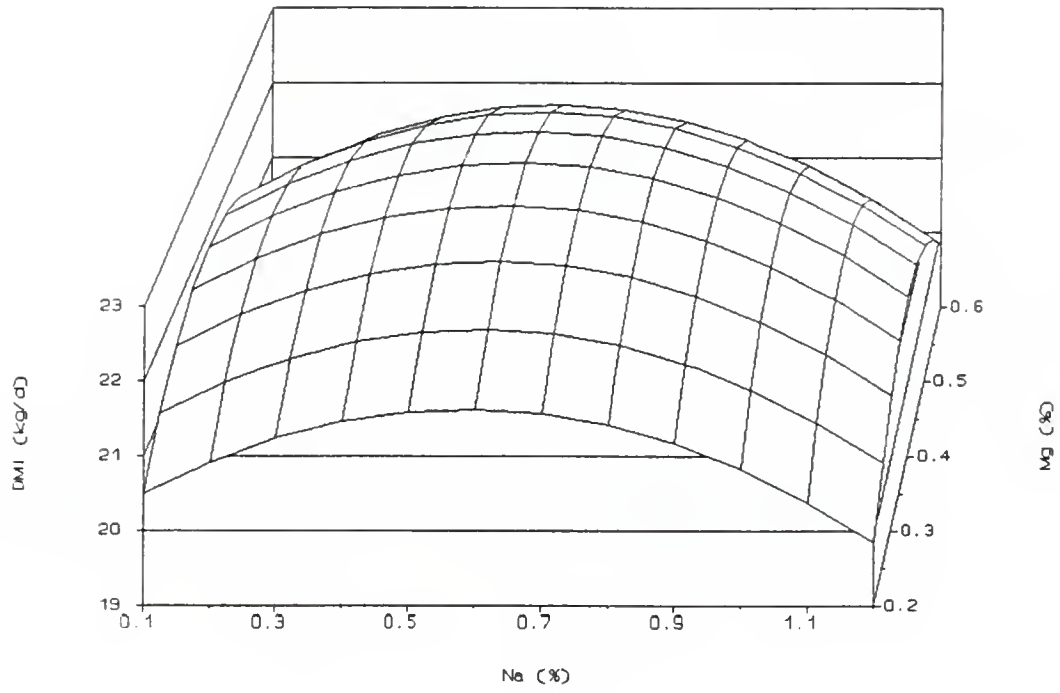


Figure 5-5. Response surfaces for dry matter intake (DMI) (A) and milk yield (B) plotted against dietary Na and Mg with all other macrominerals at their mean concentrations. Pooled standard error of means = .14 kg/d and .19 kg/d for DMI and milk yield, respectively.

B

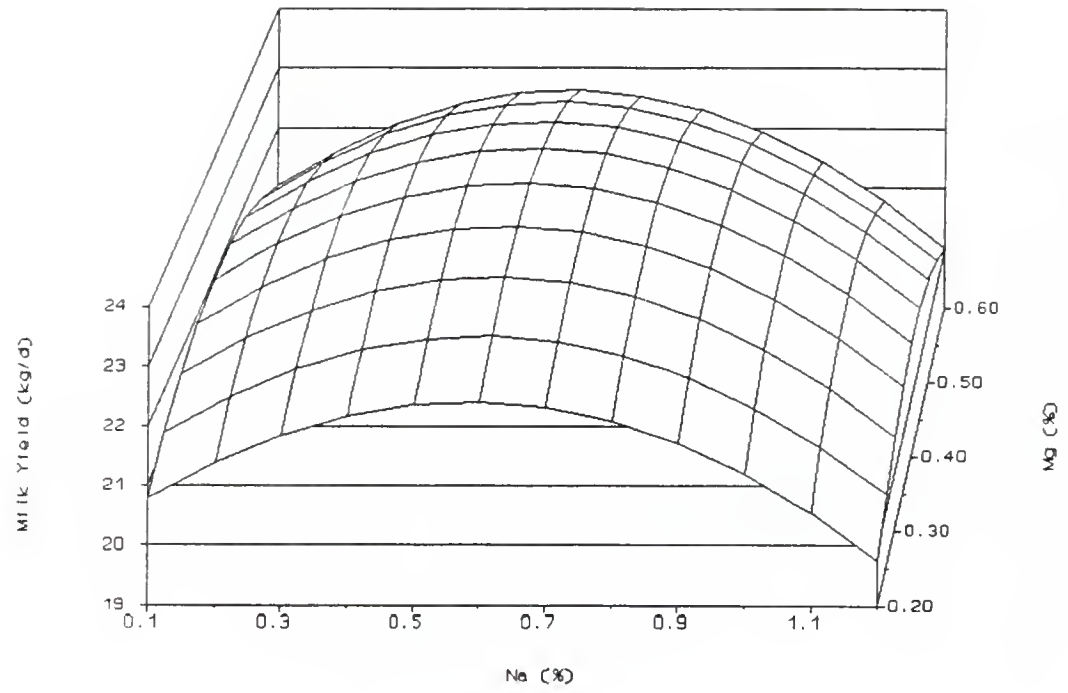


Figure 5-5--continued. Response surfaces for dry matter intake (DMI) (A) and milk yield (B) plotted against dietary Na and Mg with all other macrominerals at their mean concentrations. Pooled standard error of means = .14 kg/d and .19 kg/d for DMI and milk yield, respectively.

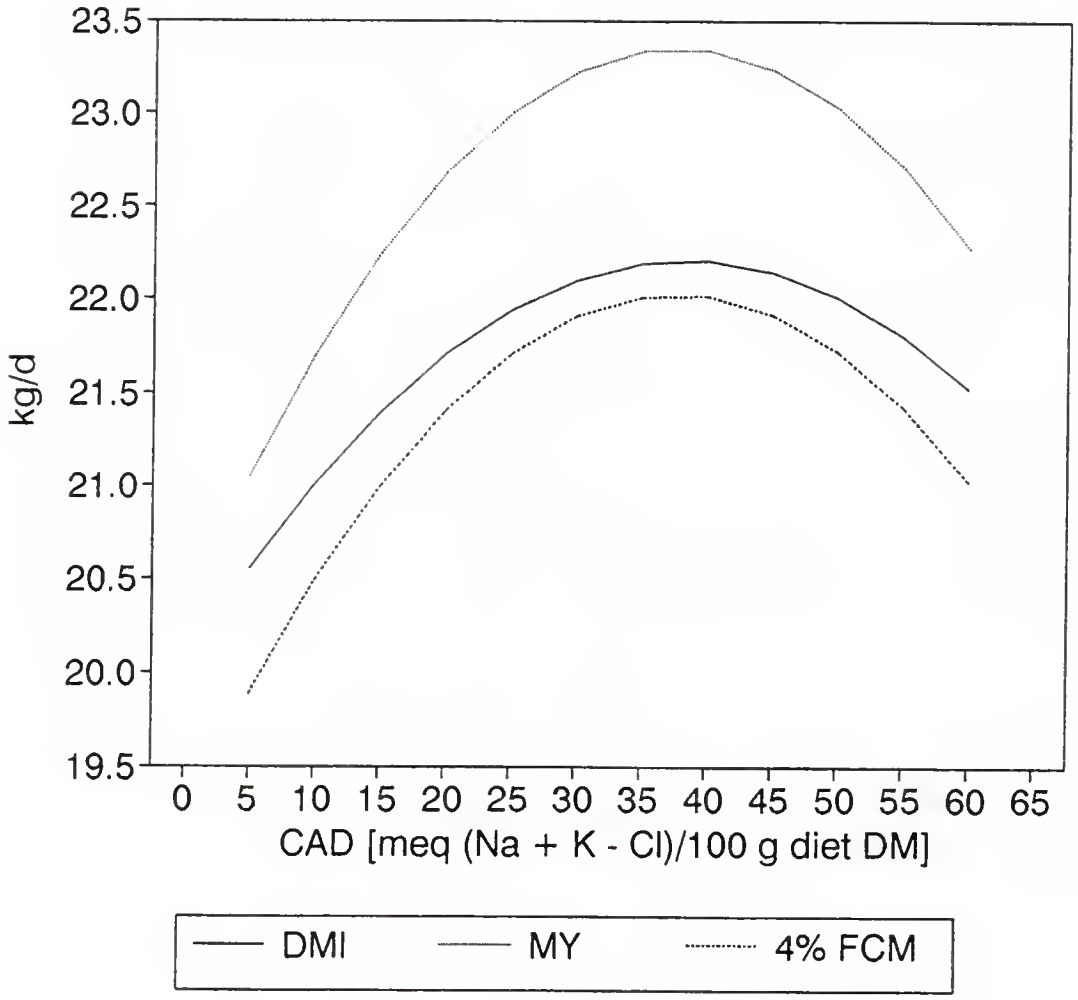


Figure 5-6. Dry matter intake (DMI), milk yield (MY) and 4% fat-corrected milk (4% FCM) yield response to cation-anion difference (CAD). Pooled standard error of means = .14 kg/d, .19 kg/d and .20 kg/d for DMI, milk yield and 4% FCM yield, respectively.

Verification and Validation of Models

Brown et al. (1980) noted that model verification and validation is an often overlooked step in development of nutrition models. Before a model can be used for prediction it must be validated, preferably with an independent data set. Unfortunately, no studies have been conducted that have simultaneously evaluated all of the macromineral interrelationships found in this analysis. Therefore, the objective was limited to identifying and quantifying macromineral interrelationships (i.e. determining the sign and magnitude of significant regression coefficients). Because no data were available to adequately verify and validate macromineral models they may not be useful for prediction. Rather, at this stage, they should be used to design future studies that address specific interrelationships. Only then can verification and validation be complete. This process is underway. For example, we recently conducted a study examining the physiological basis for a K x Cl interaction and now have data to explain how and why it may influence productive performance of the lactating dairy cow. Results from this study are presented in chapter 6 of this dissertation. The character of the K x Cl interrelationship found in this chapter was similar to that found in chapter 6. As in the present analysis, cows fed excessive Cl required additional K to prevent negative effects.

Brown et al. (1980) suggested that model publication is another often overlooked step. Rather than waiting for additional data, it was decided to present these models now to give others in the field the opportunity to examine specific interrelationships more closely and to accept or reject their significance.

Whereas there were no data to adequately evaluate macromineral models, three experiments designed to directly investigate dietary CAD for lactating dairy cattle have been published. Results from these independent data sets were compared with predicted CAD models. One study was from Tucker et al. (1988a) who fed -10, 0, +10 and +20 CAD. The other two were from West et al. (1990 and 1991) who fed +2.5, +15, +27.5 and +40 CAD in one trial and +10, +21.7, +33.4 and +45.1 CAD in another. Milk yield data from West et al. (1991) were not reported; therefore 3.5% FCM yields were used and assumed to be comparable to actual milk yields. Four percent FCM yields were not available in all independent data sets so they were not compared.

To make comparisons among regression curves it was first assumed that CAD effects from independent data sets responded in a quadratic fashion (because that was the order of the response found in the present analysis). Second order curves were then fit through least squares means from these independent studies (regressions coefficients are in Table 5-7). Figure 5-7 illustrates how these curves fit the least squares means of the independent data sets. Figure 5-8 illustrates how the independent data set curves compared with the data base predicted regression curves for DMI and MY. With these different intercepts, regression curves from the data base over-estimated DMI and MY by an average of 2.44 and 1.36; 5.96 and 3.58; and 4.53 and .34 kg/d, respectively compared with Tucker et al. (1988a), West et al. (1990), and West et al. (1991) predictions (Table 5-8). However, these deviations included animal, management and environment differences between the independent studies and those in the data base (experiment

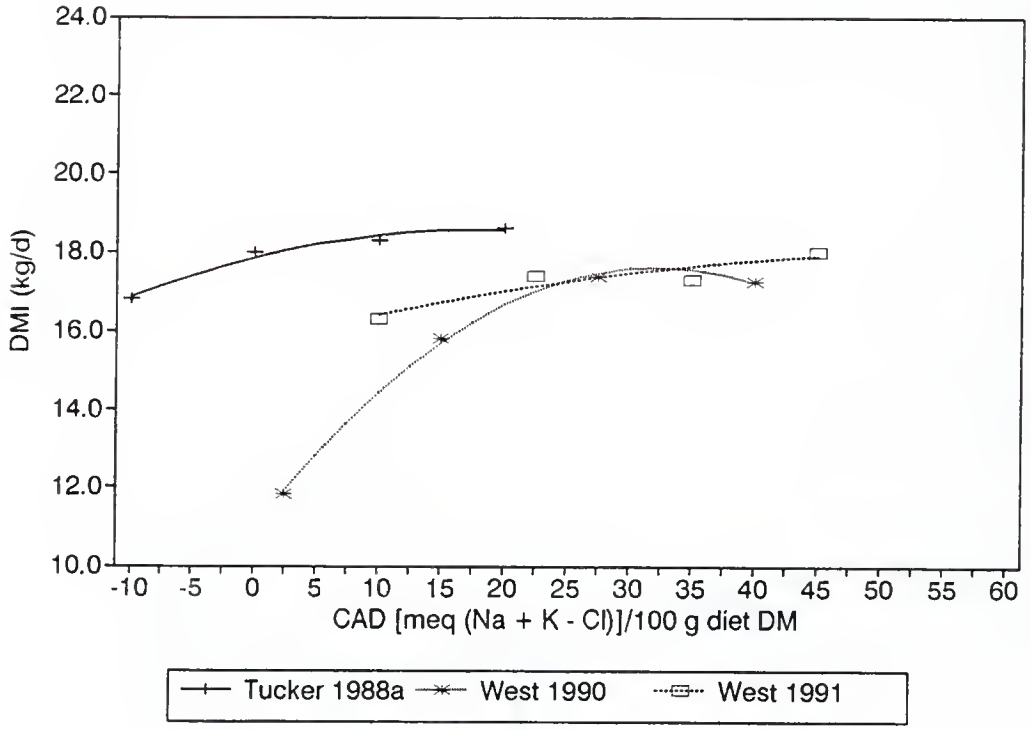
TABLE 5-7. Regression coefficients with standard errors of coefficient estimates from independent data set predicted cation-anion difference (CAD) models for dry matter intake (DMI) and milk yield (MY).[1]

	Tucker et al. 1988
DMI	= 17.865 (.149) + .0795 CAD (.02) - .00225 CAD x CAD (.001).
MY	= 19.305 (.216) + .0615 CAD (.02) - .00125 CAD x CAD (.001).
	West et al. 1990
DMI	= 10.8996 (.19) + .418 CAD (.02) - .006496 CAD x CAD (.0005).
MY	= 16.1425 (.775) + .21948 CAD (.09) - .003152 CAD x CAD (.002).
	West et al. 1991
DMI	= 15.64319 (1.093) + .08299 CAD (.09) - .00073051 CAD x CAD (.0016).
MY^2	= 20.2786 (1.475) + .1457 CAD (.12) - .00200891 CAD x CAD (.002).

[1]Fit from reported least squares means; CAD = meq (Na + K - Cl)/100 g diet DM.

[2]3.5% FCM yield. Actual MY not reported.

A



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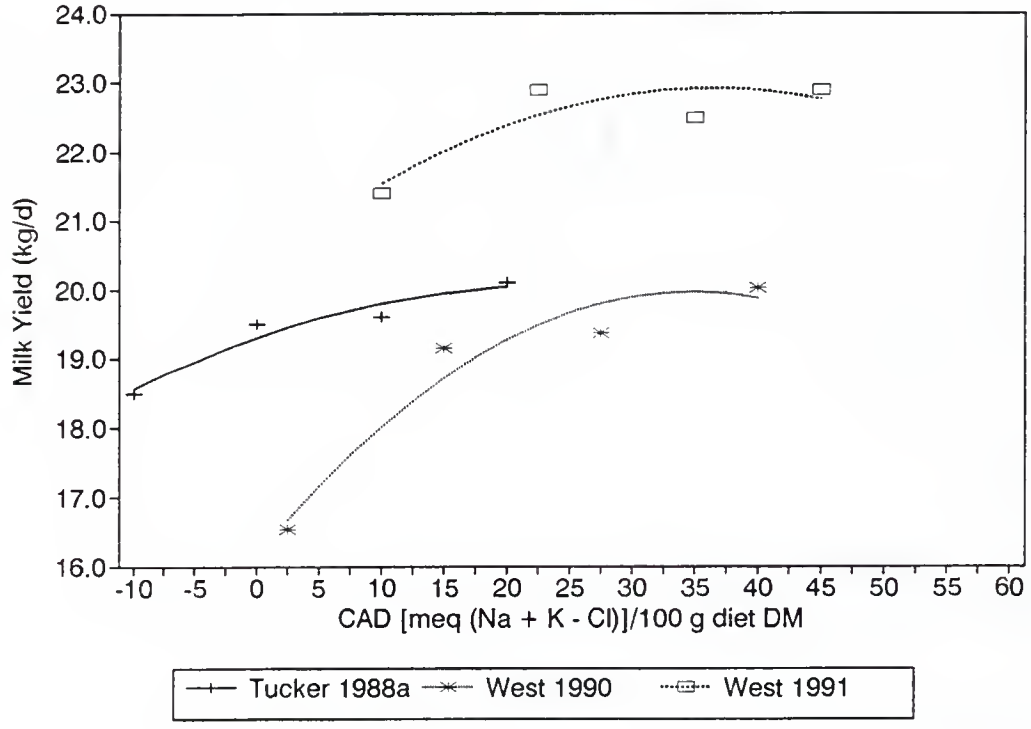
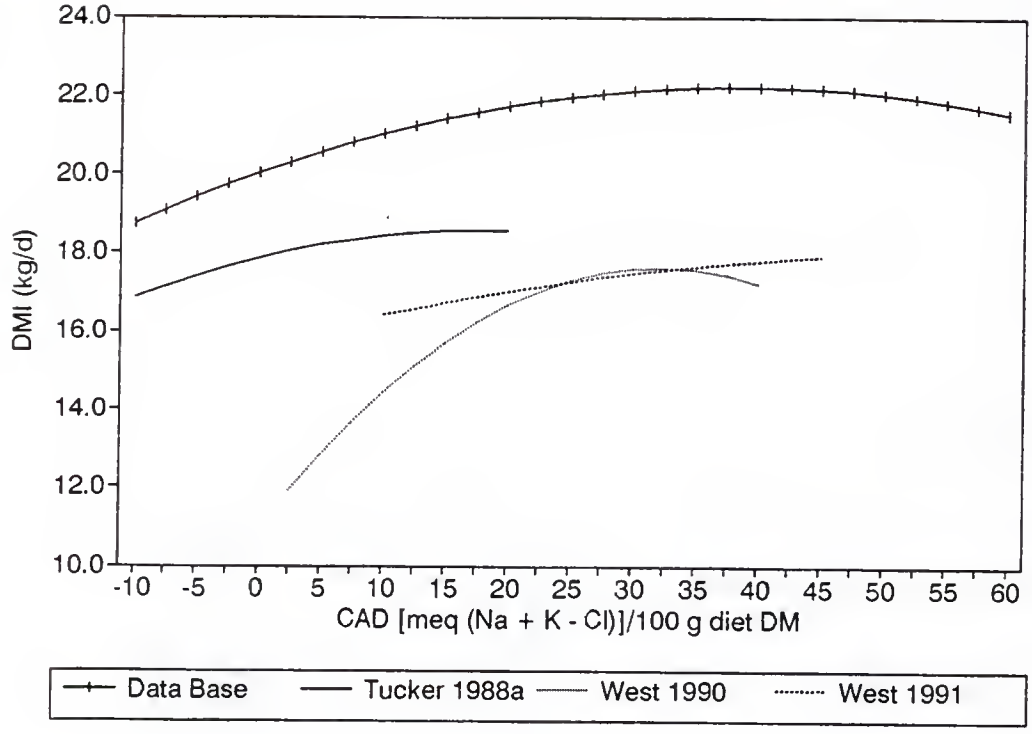


Figure 5-7. Dry matter intake (DMI) (A) and milk yield (B) least squares means and regression curves for independent data sets.

A



B

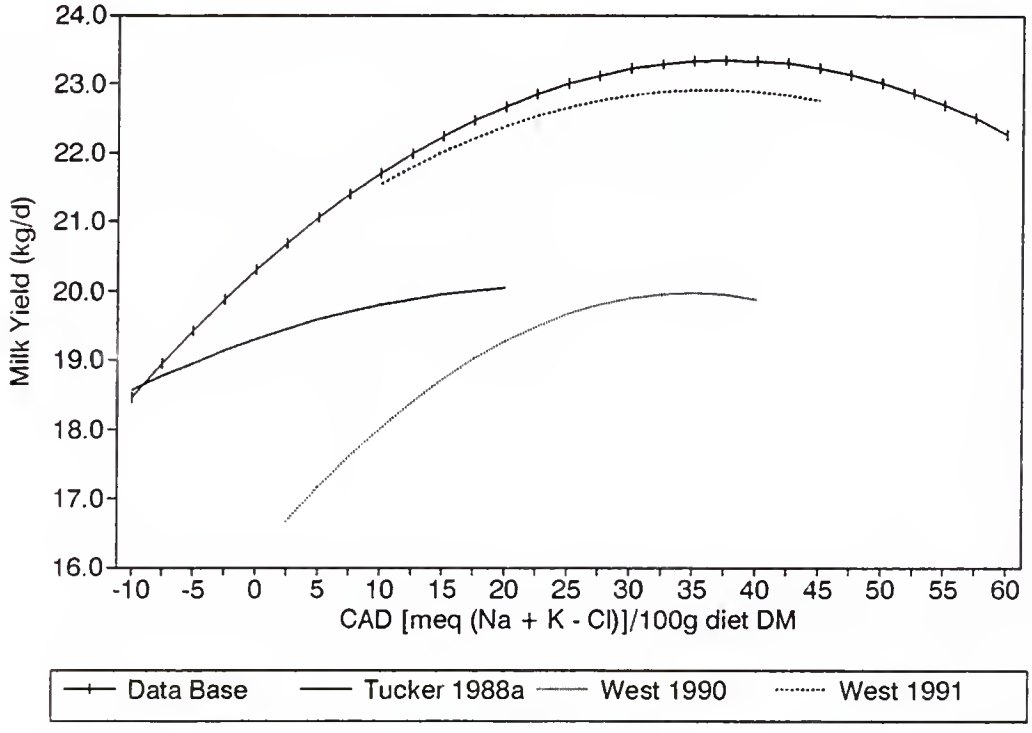
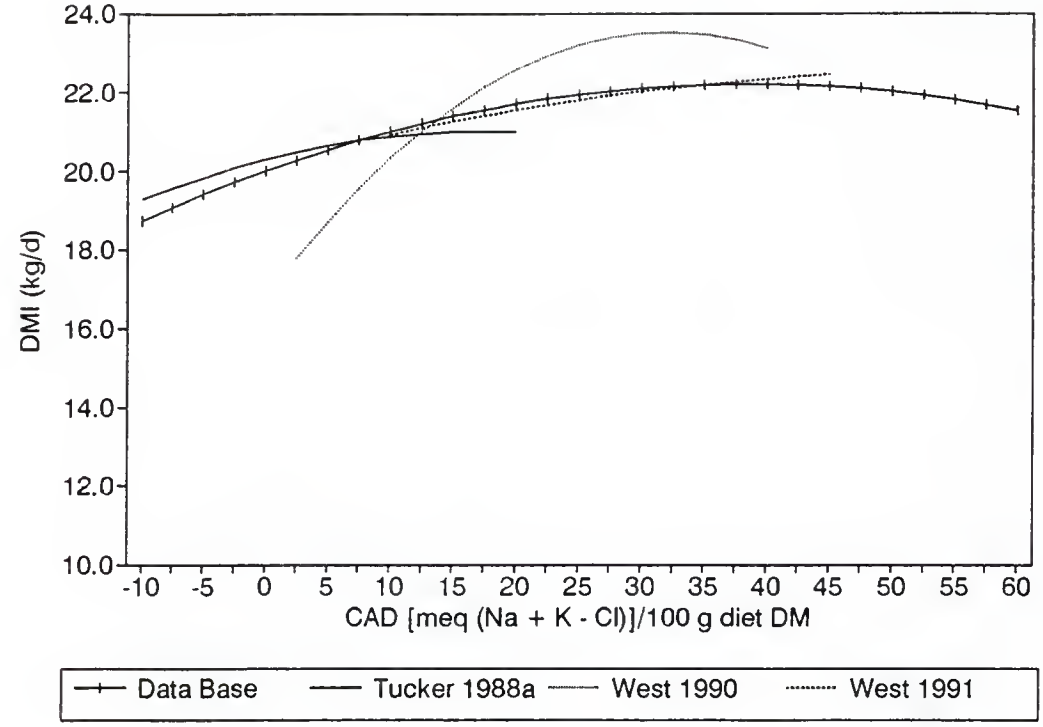


Figure 5-8. Dry matter intake (DMI) (A) and milk yield (B) regression curves for independent data sets compared to predicted CAD model regression curves.

A



B

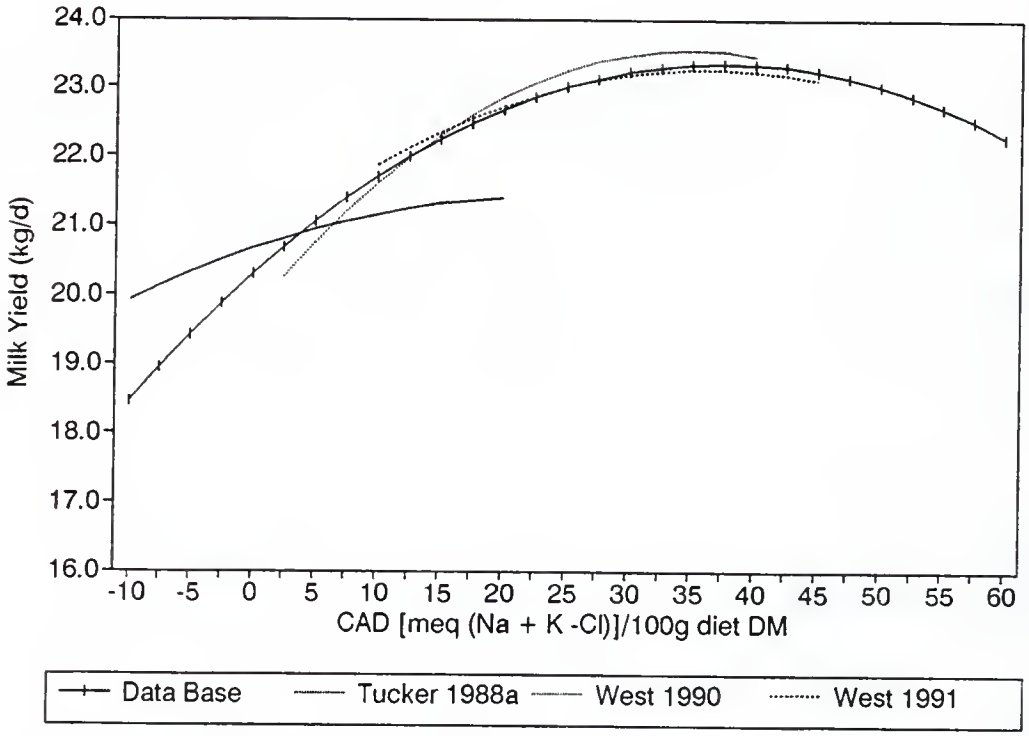


Figure 5-9. Dry matter intake (DMI) (A) and milk yield (B) regression curves from independent data sets (corrected for experiment effects) compared to predicted CAD model regression curves.

TABLE 5-8. Predicted values for dry matter intake and milk yield from cation-anion difference (CAD) models as compared to independent experiments.[1]

	Dry Matter Intake				Milk Yield			
	Predicted Values				Predicted Values			
CAD	Data Base	Independent Studies	Deviation[2]	Predicted Value + Experiment Effect	Data Base	Independent Studies	Deviation	Predicted Value + Experiment Effect
	Tucker et al. (1988)							
-10	18.74	16.85	1.89	19.29	18.46	18.57	-0.11	19.92
0	20.02	17.87	2.16	20.31	20.29	19.31	0.99	20.66
10	21.01	18.44	2.57	20.88	21.70	19.80	1.91	21.15
20	21.70	18.56	3.15	21.00	22.68	20.04	2.64	21.39
Average Deviation (experiment effect)[3]			2.44				1.36	
New Average Deviation (adjusted for experiment effect)				0.24				0.60
	West et al. (1990)							
2.5	20.30	11.9	8.40	17.86	20.69	16.67	4.01	20.25
15	21.39	15.66	5.73	21.63	22.24	18.73	3.52	22.31
27.5	22.03	17.4	4.63	23.36	23.13	19.79	3.33	23.38
40	22.20	17.11	5.10	23.07	23.34	19.88	3.46	23.46
Average Deviation (experiment effect)			5.96				3.58	
New Average Deviation (adjusted for experiment effect)				1.22				0.22
	West et al. (1991)							
10	21.01	16.4	4.61	20.93	21.70	21.54	0.17	21.87
21.7	21.79	17.1	4.69	21.63	22.80	22.50	0.30	22.83
33.4	22.17	17.6	4.57	22.13	23.31	22.91	0.40	23.24
45.1	22.14	17.9	4.24	22.43	23.23	22.77	0.47	23.10
Average Deviation (experiment effect)			4.53				0.34	
New Average Deviation (adjusted for experiment effect)				0.08				0.07

[1]Cation-anion difference = meq (Na + K - Cl)/100 g diet DM. Predicted values from data base are calculated from regression equations in Table 5-6. Predicted values from independent studies are calculated from regression equation equations Table 5-7.

[2]Deviation = predicted value from data base model minus predicted value from independent studies.

[3]Experiment effect presumed to include animal, management and environment differences between independent studies and those in data base.

effects). Correcting independent data set predictions for their associated experiment effects is analogous to adjusting the intercept of the independent studies up or down according to intrinsic experimental effects (Brown et al., 1980). Independent data set predictions corrected for specific experimental effects were plotted along with data base predicted CAD model regression curves in Figure 5-9. With these corrections, the data base CAD models predicted well. For DMI, data base CAD model and independent data set predictions differed only by an average of 2.87% (range .19 to 12.27%). For MY, they differed only by an average of 2% (range .13 to 7.94%). Absolute deviations between data base CAD model and independent data set predictions (corrected for experiment effects) ranged from .24 to 1.22 kg/d for DMI and .07 to .60 kg/d for MY (Table 5-8). A limited amount of available independent data precluded further statistical comparisons.

Deviations between predicted data base CAD models and corrected independent data set predictions were probably due to three factors. First, CAD values above +45 were not present in independent data sets. Without these higher values (i.e. above +50) an inflection point in the CAD regression line would not have been necessary and first order models (in both the data base and independent data sets) may have described the data as well as the second order models used.

The second reason for deviations in the models could have been due to extrapolations. To make comparisons with the data set of Tucker et al. (1988a), the data base regressions had to be extrapolated below the experimental range. Tucker et al. (1988a) used CAD levels to -10 but the lowest CAD included in the data base was +5.8. There were large

deviations in this range (-10 to +5 CAD), which could have been attributed to extrapolation.

A third reason for the deviations may have been due to the limited amount of data available to evaluate the models. Each of the independent studies used 4 x 4 Latin square designs with few replicates. For example, the 4 least squares means from Tucker et al. (1988a) were from three squares (12 Holstein cows); means from West et al. (1990) were from two squares (four Holstein and four Jersey cows); and means from West et al. (1991) were from four squares (16 Holstein cows). In spite of the limited amount of observations in the independent data sets, data base predictions were remarkably close to actual observations.

Conclusions

Results demonstrate the need to consider macromineral interrelationships and CAD in establishing recommended allowances for each macromineral. Macromineral models may not be ready to be used for prediction but do provide valuable empirical insight for future study. Interrelationships that had consistent effects upon several responses included dietary Na x K, Na x Ca, K x Cl, and K x Ca. Maximal intake and milk yield was achieved with .58% Na and .40% Mg. Cation-anion difference regression models indicated that +38 CAD was optimal. Predictions from CAD models were in close agreement with published data from designed CAD experiments.

CHAPTER 6
INFLUENCE OF DIETARY POTASSIUM BY CHLORIDE INTERACTION AND CATION-ANION DIFFERENCE ON PHYSIOLOGICAL RESPONSES OF LATE LACTATION DAIRY COWS

Introduction

Lactational and physiological responses are influenced by dietary concentrations of monovalent macrominerals, Na, K, and Cl (Escobosa et al., 1984; Schneider et al., 1984b; Schneider et al., 1986; Tucker et al., 1988a; West et al., 1990; Sanchez et al., 1990a, Sanchez et al., 1991) (chapter 3 and 5 in this dissertation). Not only do these cations and anions function individually, they also interrelate. Research into these interrelationships is limited but results thus far indicate that monovalent macromineral interactions have significant effects on the performance of lactating dairy cattle.

In earlier work at this station (Sanchez et al., 1990a; Sanchez et al., 1991) (chapter 3 and 5 in this dissertation) interactions between dietary K and Cl on DMI, MY, FCM yield, milk protein, weight gain, plasma Na and whole blood Mg were detected. The author is unaware of other designed experiments with lactating dairy cattle that demonstrated an interaction between K and Cl. Potential K x Cl interactions may be very important in commercial settings. Recommending how much K to feed may need to be qualified based on the relative amount of dietary Cl. A specific macromineral recommendation, like that given by NRC (1989), may need to be adjusted for interactions.

Close examination of the K x Cl interaction (Sanchez et al., 1990a; Sanchez et al., 1991) (chapter 3 and 5 in this dissertation) implied that optimum concentrations of dietary K and Cl for maximum lactational performance were achieved when they were coupled together or changed in unison. The physiological basis of these responses is currently unknown. Belgium researchers (Paquay et al. 1969a) proposed one theory. Using survey data from metabolism trials they found a close positive correlation between K and Cl in urine. Their hypothesis was that because ruminants consume large quantities of K from forage and because dietary K is absorbed nearly completely from the gastrointestinal tract, K must be eliminated in the urine. Because electrical neutrality of biological fluids is necessary (Stewart, 1981), a reciprocal ion of opposite charge must accompany K in urine. Chloride, the anion in greatest concentration in the plasma (Lunn and McGuirk, 1990) most often serves this purpose. If there is excess K relative to Cl (or vice versa, excess Cl relative to K) in the diet, the normal process of urinary excretion of cations and anions can be perturbed. This in turn may impact acid-base status. Lunn and McGuirk (1990) suggested that the relative excretion of cationic and anionic electrolytes mediate the regulation of acid-base status. Homeostasis of extracellular fluid H^+ concentration $[H^+]$ is critical due to the impact $[H^+]$ ions have on enzyme function and biological reactions, membrane transport and osmotic balance, O_2 and CO_2 transport, bone mineral metabolism, and kidney function (Madshus, 1988; Morris, 1986; Stewart, 1981). Small changes in acid-base status can cause significant changes in productive performance (Kronfeld, 1976).

Using a quantitative approach, Stewart (1981) found that [H^+] is one of a number of variables that ultimately depends upon the concentration of three independent variables in blood: (1) the plasma strong ion difference, meq (Na + K - Cl)/L; (2) weak acids (proteins); and, (3) the partial pressure of CO_2. Intestinal and renal excretion affects the strong ion difference, which in turn affects H^+ concentration. Potassium x chloride interactive effects on lactational performance of dairy cattle may be induced by the effects that strong ions have on acid-base status. Animal nutritionists have been studying the strong ion difference concept under the name dietary cation-anion balance (Mongin, 1981; Tucker et al., 1988a; West et al., 1991), or more specifically, dietary cation-anion difference (CAD) which is expressed as meq (Na + K - Cl)/100 g diet DM. In earlier studies [(Sanchez et al., 1990a; Sanchez et al., 1991) (chapter 3 and 5 in this dissertation), Tucker et al. (1988b), and West et al. (1990 and 1991)] it was found that CAD values below +20 meq (i.e., relatively high dietary concentrations of Cl relative to Na and K), and above +50 meq (i.e., relatively high dietary concentrations of Na and or K relative to Cl), reduced milk production of dairy cows.

Tucker et al. (1988a and 1988b), Sanchez et al., (1990a) (chapter 3 in this dissertation) and West et al. (1990) reported effects of CAD on blood acid-base status. Interactive effects between K and Cl on milk production responses were thought to be due to alterations in blood acid-base status and mineral metabolism. This has yet to be substantiated. Further, it is not known how rapidly physiological responses to varying dietary K and Cl occur and how long and to what

extent compensatory mechanisms might respond. In the report of Tucker et al. (1988b), urinary responses to differing dietary Na and Cl and CAD were immediate (within minutes to hours after introducing new diets). Others have claimed that the bovine kidney responds rapidly to differing dietary concentrations of electrolytes by excreting varying amounts of cations and anions (Lunn and McGuirk, 1990). There are, however, no collected data from the lactating cow which provide a quantitative definition of the acute postprandial physiological response to interactions between dietary K and Cl. Therefore, the objectives of this experiment were to determine postprandial physiological responses of lactating dairy cows to dietary K, Cl, K x Cl interactions, and CAD.

Materials and Methods

Experimental Design

The design used was a replicated 4 x 4 Latin square split-plot in time. An extra period (period 5), in which cows continued on period 4 treatments, was used to balance for and account for carryover effects (Cochran and Cox, 1957). Eight late lactation (233 DIM) Holstein cows were blocked into two groups (squares) according to beginning BW (block 1 average = 1044 kg; block 2 average = 1283 kg) and randomly assigned to treatment sequences. Cows were adapted to basal diet (Tables 6-1 and 6-2) for 1 wk prior to initiation of the experiment. The experimental lasted 7 d.

Treatments

Treatments were arranged as a 2 x 2 factorial and consisted of two

dietary concentrations each of K and Cl (Table 6-1). The first treatment was formulated with low K, low Cl (1.11% K, .43% Cl; LK:LCl; basal diet); the second with high K, low Cl (1.72% K, .44% Cl; HK:LCl); the third with low K, high Cl (1.07% K, .91% Cl; LK:HCl); and, the fourth with high K, high Cl (1.80% K, .90% Cl; HK:HCl). Concentrations of K_2CO_3, $CaCl_2$, $CaCO_3$ and SiO_2 were varied in treatment formulations to obtain different dietary concentrations of K and Cl but maintain equal concentrations of other nutrients. Diets were mixed as a TMR, consisting of corn silage and concentrate. Formulations were designed to meet or exceed established nutrient recommendations of lactating dairy cattle (NRC, 1989).

Housing and Care

Cows were kept at the University of Florida Dairy Research Unit, in tie-stalls in an enclosed flat barn and milked twice-daily at 0600 and 1600 h. They were allowed to exercise daily in a sand lot between 2000 and 0600 h. Rations of corn silage and concentrate were mixed in an electronic feeding system (American Calan, Inc., Northwood, NH) each afternoon and weighed into feed tubs. Animals were fed once daily at 0600 h. Amounts fed were restricted to approximately 90% of ad libitum (on an as fed basis estimated during preliminary period) to ensure total consumption of dietary treatments. Water was provided ad libitum via cup waters in each stall.

Sample Collection and Analysis

Beginning 3 d prior to the first experimental period and

TABLE 6-1. Ingredient composition of treatment diets (% of diet DM).

Ingredient	Dietary Treatments[1]			
	LK:LCl CAD 39	HK:LCl CAD 58	LK:HCl CAD 25	HK:HCl CAD 45
Corn silage	45	45	45	45
Ground yellow corn	25	25	25	25
Corn distillers dried grains	11	11	11	11
Soybean meal	14	14	14	14
Calcium carbonate	1	1	...	...
Dicalcium phosphate	.15	.15	.15	.15
Vitamin-mineral premix[2]	.75	.75	.75	.75
Trace mineralized salt[3]	.25	.25	.25	.25
Na_2CO_3	.75	.75	.75	.75
K_2CO_3	...	1.50	...	1.50
KCl	.10	.10	.10	.10
MgO	.25	.25	.25	.25
$CaCl_2$	.00	.00	1.10	1.10
SiO_2 (filler)	1.75	.25	1.65	.15
Total	100	100	100	100

[1]LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl); CAD = cation-anion difference, meq (Na + K - Cl)/100 g diet DM.

[2]Contained Ca 22%, P 13%, Mg 2%, S 1%, Mn .22%, Zn .33%, Cu .12%, I .007%, Se .003%, Co .0002%, Vitamin A 110,000 IU/kg, Vitamin D_3 99,000 IU/kg, Vitamin E 330 IU/kg.

[3]Contained NaCl 92%, Mn .25%, Fe .2%, Cu .033%, I .007%, Co .0025%.

continuing daily throughout the experiment, cows were dosed with a gelatin capsule containing 10 g of Cr_2O_3 via balling gun twice daily following milking (twice daily). Fecal grab samples were taken after dosing beginning on d 1 and then continuing daily throughout the experiment.

Indwelling urethral and jugular catheters were placed in each cow on the afternoon before the start of each experimental period. Jugular catheters were kept patent with 200 U/ml ammonium heparin in sterile physiological saline (.9% NaCl).

Samples of TMR were taken thrice weekly and pooled within period. Morning and evening fecal samples were pooled within cow and composited on a dry basis. Feed and feces were dried (100 °C) for 24 hr and then ground through 2-mm screen (Thomas Wiley Mill, model 4, Thomas Scientific, Philadelphia, PA). Samples of feed and feces were ashed (550 °C) overnight. Ash was dissolved in 3 N HCl, diluted and analyzed for Na, K, Ca and Mg by atomic absorption spectrophotometry (model 5000, Perkin Elmer, Inc., Norwalk, CT). Feed and fecal Cl concentrations were determined by dissolving samples in .4 N HNO_3 and 40% glacial acetic acid, shaking vigorously for 1 h and then centrifuging at 12,000 x g for 10 minutes. Chloride ions in harvested supernatant were measured by coulometric titration (Haake Buchler Instruments, Inc., Saddlebrook, NJ). Other nutrients were measured by commercial lab (DHIA forage laboratory, Ithaca, NY). Feces were analyzed for Cr according to Williams et al. (1962) and apparent mineral digestion coefficients were calculated by marker ratio (Schneider and Flatt, 1975).

Sampling of blood and urine was done in two phases. Phase I samples were collected over several h on the first d of each period. Phase II samples were collected daily at 4 h postfeeding.

Phase I blood samples were collected beginning at milking and feeding (h 0) and then .5, 1, 1.5, 2, 2.5, 3, 4, 5, 7, 10 and 13 h postfeeding. At each blood sampling time, two jugular samples were collected. One 5 ml sample (collected in a plastic syringe coated with 20 U/ml sodium heparin) of whole blood kept anaerobic was immediately analyzed for pH and ionized Ca (NOVA 8 analyzer, NOVA Biochemicals, Waltham, MA). An additional 15 ml jugular sample was dispensed into two plastic tubes containing ammonium heparin (20 U/ml). One tube (10 ml) was centrifuged for 20 min (2500 x g) to harvest plasma. Plasma was transferred to 7 ml plastic tubes and stored frozen (-10 ° C) for later analyses of Na, K, Cl and total CO_2 (total CO_2 was assumed to represent blood HCO_3^-; Kleinman and Lorenz, 1989). Remaining red blood cells were washed and re-centrifuged three times with isotonic choline chloride and frozen until analysis for K. Choline chloride was made isotonic to physiological saline (.9% NaCl, .1538 M) by adding 21.47 g choline chloride to 1 L of distilled water. The second sample of jugular blood (5 ml) was used to measure hematocrit and total percent plasma protein (Schuco Clinical Refractometer, model 5711-2020, Schuco Division of American Caduceus Industries, Inc., Carle Place, NY).

Phase I urine samples were collected at 0, 1, 2, 3, 4, 7, 10, and 13 h postfeeding. At each urine sampling time, a minimum of 100 ml urine was collected directly from catheter tubing into plastic beakers. Samples were split into two parts. One part was placed into a 14 ml

plastic tube (non- acidified sample) and the other part into 168 ml plastic bags containing .5 ml of 50% (w/v) H_2SO_4. The non acidified urine samples were analyzed immediately for pH using a digital pH/millivolt meter (Model 611, Orion Research, Cambridge, MA) and stored frozen (-10 °C) for subsequent Cl and creatinine analyses. The acidified samples were transferred to 14 ml plastic tubes and stored frozen (-10 ° C) for subsequent ammonium, Na, K, Ca and Mg analyses.

At the end of sample collection on d 1, urethral and jugular catheters were removed. On d 2 through 7 of each experimental period, blood and midstream urine samples were collected daily via jugular venipuncture and vulva stimulation, respectively, at 4 h postfeeding and handled in the same manner as d 1 samples.

Plasma concentrations of Na, K and Cl, and HCO_3^- (as total CO_2) were determined on a 664 Fast 4 System (CIBA-CORNING Diagnostics Corp., Medfield, MA). Urine concentrations of Na, K, Ca and Mg were measured via atomic absorption spectrophotometry (Model 5000, Perkin Elmer, Inc., Norwalk, CT). Urine Cl was determined by coulometric titration (Haake Buchler Instruments, Inc., Saddlebrook, NJ). Red blood cell K was assayed via atomic absorption spectrophotometry (model 5000, Perkin Elmer, Inc., Norwalk, CT) after digesting 1 ml sample of blood in 3 ml concentrated HNO_3 in 30 ml glass tubes placed on a hot plate for 20 min. Urine creatinine and urine ammonium were determined colorimetrically according to methods described by Henry et al. (1974) and Chaney and Marbach (1962), respectively.

Feed intake, milk yield and milk composition were measured daily. One milk sample split into two subsamples was collected at each milking.

One subsample was preserved with $K_2Cr_2O_7$ capsules and assayed for fat, protein, lactose, and total solids (DHIA, Blacksburg, VA). The unpreserved sample was first assayed for pH then stored frozen (-10 ° C) in 14 ml plastic tubes for later mineral analyses. After thawing, milk samples were pooled within cows and d and assayed for Na, K, Ca, and Mg according to methods used for red blood cells. Milk Cl was determined after precipitating in an acid $ZnSO_4$ (somogyi precipitate, Cotlove, 1963) using the same method described for feeds.

On the last 3 mornings of each period and 3 d before period one, BW were recorded (prior to milking) to measure weight changes and block animals. Respiration (breaths/min), and rectal temperature were recorded daily at 4 h postfeeding. Cows were observed carefully for visually detectable differences in pattern of feed intake, urination, and health. Data were removed from one cow treated for displaced abomasum (d 5 through 7 in period three), from one cow with acute dietary indigestion (d 7 in period three and d 1 through 4, period four), and from one cow with mastitis (d 6 through 7, period 4 and d 1 though 4 period 5).

Statistical Analysis

Data were analyzed by the method of least squares ANOVA, using general linear model (GLM) procedures of SAS (1985). Statistical analysis was completed in two stages because single-period treatment carryover effects were confounded with time effects if data were analyzed as a split-plot. In stage one, GLM procedures of SAS (1985) were performed for each time point. In these models independent

variables were square, cow(square), period, treatment, and treatment carryover effects. Treatment least squares means for each time point were generated and used in the second stage of the analysis to investigate treatment and treatment x time (continuous independent variable) interactions. Single-period treatment carryover effects were accounted for (if they existed) but were not quantitatively estimated in the overall statistical analysis. Treatment effects were separated into two categories. The first category partitioned treatment effects into main effects of dietary K, main effects of dietary Cl, and K x Cl interaction. The second category partitioned treatment effects into linear and quadratic effects of CAD.

The two following analyses were conducted: (1) an hourly analysis (Phase I) consisting of data from 0 through 13 h postfeeding on d 1, and (2) a daily analysis (Phase II) consisting of daily data collected at 4 h postfeeding on d 1 through 7. Error terms for hypothesis testing of treatment, time and interactions of treatment x time were the sum of mean squares from the separate time point analysis of variance tables generated in stage one. Error degrees of freedom were likewise from stage one (from associated mean square errors for each time point).

Results and Discussion

Ingredient and analyzed nutrient composition of treatments are shown in Tables 6-1 and 6-2. Dietary K and Cl concentrations were close to formulated values and ranged from 1.07 to 1.80% K and .43 to .91% Cl. Calculated CAD from these analyses ranged from +25 to +58. Acid detergent fiber was below that recommended by NRC (1989) but NDF was

TABLE 6-2. Analyzed chemical composition of dietary treatments (% of diet DM).

Item	LK:LCl CAD 39	HK:LCl CAD 58	LK:HCl CAD 25	HK:HCl CAD 45
NE_L, Mcal/kg[1]	1.63	1.65	1.63	1.65
CP, %	17.7	17.9	17.4	16.8
UIP, % of CP[2]	37	37	37	37
ADF, %	17.4	16.4	18.7	17.1
NDF, %	37.5	38.2	39.9	37.6
Ca, %	.79	.79	.78	.76
P, %	.59	.52	.54	.56
Mg, %	.35	.34	.35	.36
S, %	.25	.24	.25	.25
Na, %	.53	.60	.54	.55
K, %	1.11	1.72	1.07	1.80
Cl, %	.43	.44	.91	.90
CAD[3]	39	58	25	45

[1]Value calculated from chemical analysis.

[2]Value calculated from NRC (1989).

[3] CAD = meq (Na + K - Cl)/100g diet DM.

adequate. Results of dependent variables that were affected by both treatment and time ($P < .1$) are shown in Figures 6-1 through 6-9. Results for dependent variables that were affected only by treatment were averaged across time and are presented in Tables 6-3 through 6-8. Least squares ANOVA of all pH data was performed on values transformed to $[H^+]$ first. Values were transformed back to pH for ease of interpretation, but pH data were not evaluated statistically (Murphy, 1982).

Phase I--Hourly Sampling

Blood acid-base status and mineral metabolism. Effects of dietary treatments on blood variables during Phase I are presented in Table 6-3. Blood $[H^+]$ decreased with increasing dietary K ($P < .05$) and increasing dietary CAD ($P < .01$) and tended to increase ($P < .1$) with increasing dietary Cl. Time did not influence blood H^+ ($P > .1$). Figure 6-1 illustrates blood ionized Ca responses to treatment and h postfeeding. Ionized Ca responded curvilinearly through time postfeeding (cubic h effect, $P < .01$), and was elevated by increasing dietary Cl ($P < .05$). Ionized Ca responses to Cl and CAD depended upon time postfeeding. No differences were observed until 5 h postfeeding. After that time, ionized Ca appeared to be greater for cows fed diets with high Cl and low CAD. This resulted in h x Cl ($P < .01$) and h x CAD ($P < .1$) interactions.

Based on results in Figure 6-1 and Table 6-3, it appeared that most of the effect on acid-base status was due to LK:HCl. However, because contrast comparisons between this diet and the others (pooled

TABLE 6-3. Effects of dietary K, Cl and cation-anion difference (CAD) on blood variables not affected by dietary treatment by h interactions.[1]

Variable	Dietary Treatments[2,3] LK:LCl CAD 39	HK:LCl CAD 58	LK:HCl CAD 25	HK:HCl CAD 45	SEM[4]
H^+, neq/L[a,b,c]	35.74	35.41	37.32	35.71	.78
pH[5]	7.447	7.451	7.428	7.447	
Plasma HCO_3^-, meq/L	23.61	23.54	22.82	23.54	.74
Plasma Na, meq/L	143.60	143.15	143.47	143.80	.72
Plasma Cl, meq/L[c,d]	104.62	104.19	106.06	105.19	.91
Red blood cell K, meq/L[e]	16.46	16.16	16.65	15.91	.46
Hematocrit, %	28.78	28.75	28.71	29.10	.63
Plasma protein, mg/dl	7.72	7.69	7.55	7.58	.17

[1]Least squares means pooled across Phase I sampling times (h 0 through 13, d 1 sampling times).

[2]LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl).

[3]CAD = meq (Na + K - Cl)/100 g diet DM.

[4]Standard error of the mean.

[5]Calculated from H^+ concentration; not evaluated statistically.

[a]Difference due to dietary K ($P < .05$).

[b]Difference due to dietary Cl ($P < .1$).

[c]Difference due to dietary CAD ($P < .01$)

[d]Difference due to dietary Cl ($P < .05$).

[e]Difference due to dietary K ($P < .1$).

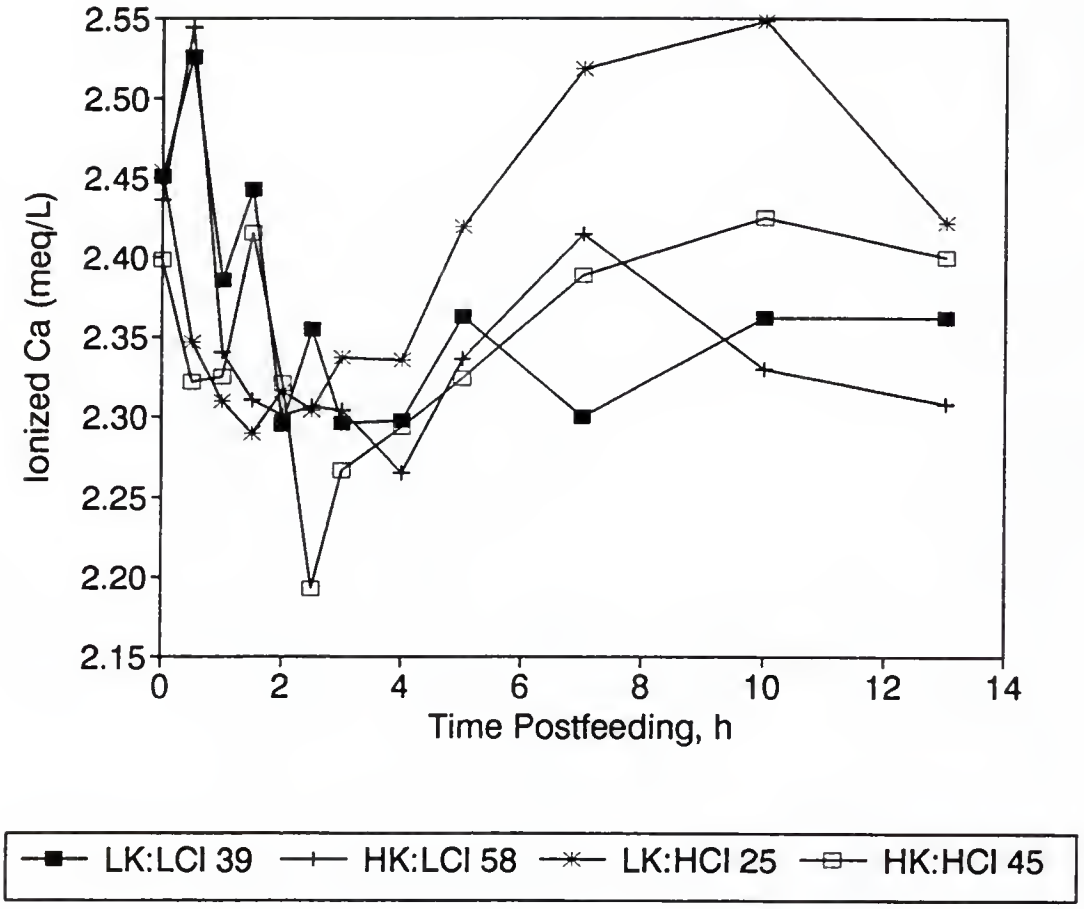


Figure 6-1. Least squares means of ionized Ca during d 1. LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl). Values for CAD are next to treatment designations in figure legend. Standard error of the mean = .08 meq/L.

average) was not decided upon a priori they were not tested. Coppock et al. (1982), Escobosa et al. (1984) and Tucker et al. (1988b) also reported symptoms of metabolic acidosis when high Cl diets were fed to lactating cows. These authors did not measure ionized Ca. Oetzel et al. (1988) and Wang and Beede (1992) reported increased ionized Ca when diets high in Cl and S (as ammonium salts) were fed to dry cows. Wang and Beede (1992) suggested that anionic diets induced subclinical metabolic acidosis which led to increased blood ionized Ca.

Plasma K tended to increase with increasing dietary K ($P < .1$) but was unaffected by CAD (Table 6-3). Time postfeeding influenced plasma K response to dietary treatments (cubic h effect, $P < .05$). Treatment effects on plasma K were more visible 5 h after feeding (Figure 6-2). Red blood cell K tended to decrease with increasing dietary K ($P < .1$). Time postfeeding did not influence red blood cell K ($P > .1$). Plasma Cl increased with increasing dietary Cl ($P < .05$), and decreased with increasing CAD ($P < .01$), but did not change over time ($P > .1$). Escobosa et al. (1984) reported increases in plasma Cl by feeding 1.65% Cl. Tucker et al. (1988b) did not find consistent differences in plasma Cl when comparing diets with .34 vs. .86% Cl. High Cl diets of Tucker et al. (1988b) were much higher in CAD than the high Cl diet of Escobosa et al. (1984) (+56 vs. -19). No effects of dietary K, Cl or CAD on plasma Na, plasma HCO_3^-, plasma protein or blood hematocrit were observed ($P > .1$) in Phase I of this experiment.

Urinary cation and anion concentrations. Urinary mineral concentration varies depending upon urine volume. By expressing urine

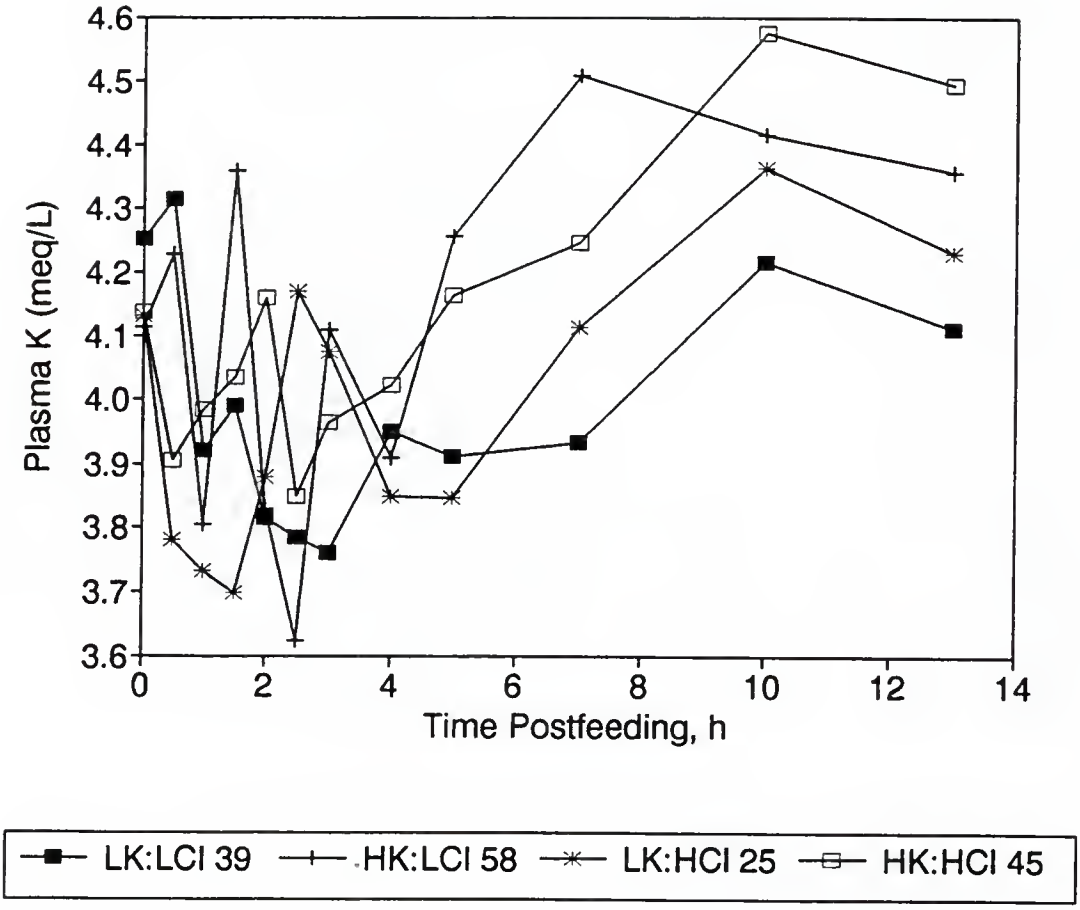


Figure 6-2. Least squares means of plasma K during d 1. LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl). Values for CAD are next to treatment designations in figure legend. Standard error of the mean = .129 meq/L.

minerals as a proportion of urine creatinine concentration, much of the effect of changes in urine mineral concentration associated with urine volume can be eliminated (Tucker et al., 1988b; Wang and Beede, 1992; Lunn and McGuirk, 1990). The ratio of urinary mineral concentration to urinary creatinine concentration can be estimated from individual urine samples and was found to be a satisfactory indicator of urinary mineral excretion in ruminants as compared with fractional clearance (McKinnon, 1984, cited by Lunn and McGuirk, 1990; Schneider et al., 1988; Tucker et al., 1988b, Wang and Beede, 1992).

Effects of dietary K, Cl, and CAD on urine variables in Phase I are presented in Table 6-4. Cows fed high Cl ($P < .05$) and low CAD (linear CAD effect, $P < .05$) had greater urinary [H^+]. Recall that cows fed high Cl also had greater blood [H^+]. Apparently cows were excreting blood H^+ ions into urine. Tucker et al. (1988a) and Tucker et al. (1988b), noted increased urine H^+ with low CAD. It appeared from the current study that urinary [H^+] was greatest with LK:HCl but this was not tested statistically.

Urinary Na/creatinine ratio responded curvilinearly over time (quadratic h effect, $P < .01$; Figure 6-3); response to treatment depended upon time postfeeding (h x K interaction, $P < .05$). At early sampling times no differences due to dietary K were evident; but after 4 h postfeeding, urinary Na/creatinine appeared to increase with increasing dietary K. Increases in urinary Na due to increasing dietary K and CAD are not understood. Lomba et al. (1969) reported a .176 correlation (nonsignificant) between urinary Na and K intake. O'Connor (1987) did not detect increased urinary Na with increasing dietary K

TABLE 6-4. Effects of dietary K, Cl and cation-anion difference (CAD) on urine variables not affected by dietary treatment by h interactions.[1]

Variable	Dietary Treatments[2,3] LK:LCl CAD 39	HK:LCl CAD 58	LK:HCl CAD 25	HK:HCl CAD 45	SEM[4]
H^+, neq/L[a,b]	5.78	2.87	15.33	9.92	4.58
pH[5]	8.238	8.542	7.814	8.003	
$NH4_4^+$/Creatinine, mmol/mmol	1.06	1.62	1.72	1.61	1.39
Ca/Creatinine, mmol/mmol[b,c]	.213	.107	.367	.158	.103
Mg/Creatinine, mmol/mmol	8.73	9.11	8.82	7.66	1.192

[1]Least squares means pooled across Phase I sampling times (h 0 through 13, d 1 sampling times).

[2]LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl).

[3]CAD = meq (Na + K - Cl)/100g diet DM.

[4]Standard error of the mean.

[5]Calculated from H^+ concentration; not evaluated statistically.

[a]Difference due to dietary Cl ($P < .05$).

[b]Difference due to linear effect of CAD ($P < .05$).

[c]Difference due to dietary K ($P < .1$)

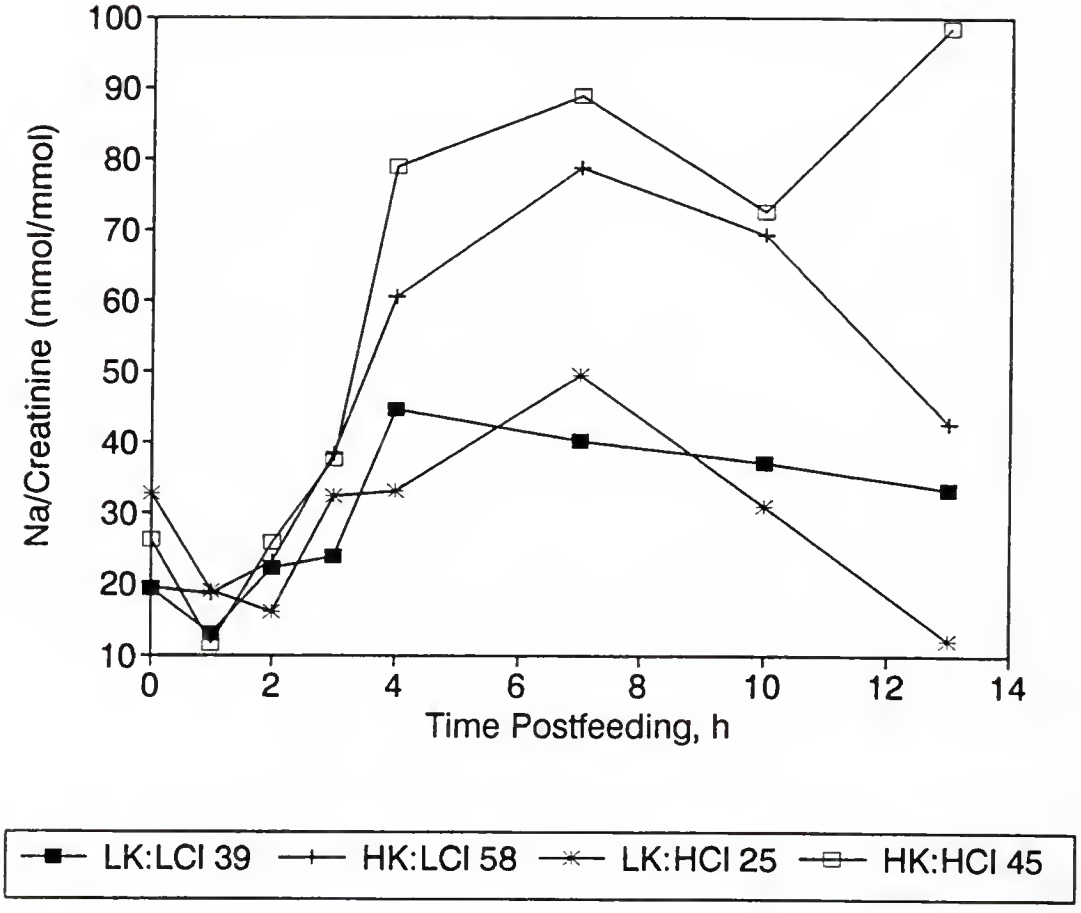


Figure 6-3. Least squares means of urine Na/creatinine ratio during d 1. LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl). Values for CAD are next to treatment designations in figure legend. Standard error of the mean = 8.74 mmol/mmol.

(1.65 vs. .87% K), but diets in that study had .94% Cl. Dietary CAD did not affect urinary Na/creatinine ($P > .1$). Tucker et al. (1988a) reported an increase in urinary Na concentration with increasing CAD (from -10 to +20), but increased urinary Na was associated with diets with higher Na concentrations.

Urinary K/creatinine responses were similar to urinary Na/creatinine responses (Figure 6-4). Both cations were affected by h x K interaction ($P < .01$ for urinary K/creatinine), however urinary K/creatinine increased through time linearly (linear h effect, $P < .01$) instead of curvilinearly. A significant linear h x CAD interaction ($P < .05$) also affected urinary K/creatinine. High urinary K with increasing dietary K has been observed previously (St. Omer and Roberts, 1967). Paquay et al. (1969b) reported .903 correlation between K intake and urinary K. O'Connor (1987) found increased fractional excretion of K with increasing dietary K.

Urinary Cl/creatinine response followed a curvilinear trend through time (cubic h effect, $P < .1$). However, urinary Cl/creatinine response to dietary Cl depended upon time postfeeding (h x Cl interaction, $P < .01$) and dietary K x Cl interaction (h x K x Cl interaction, $P < .05$). The h x K x Cl interaction was due to the simple effect of dietary K at the high concentration of Cl. After 4 h postfeeding, cows fed HK:HCl had urine with 2- to 3-fold greater Cl/creatinine than cows fed LK:HCl (Figure 6-5). This interaction effect on urinary Cl/creatinine is difficult to visualize in Figure 6-5. In Figure 6-6, increasing dietary Cl from .43 to .91% did not affect urinary Cl/creatinine at h 0 (dotted lines). However, by 13 h

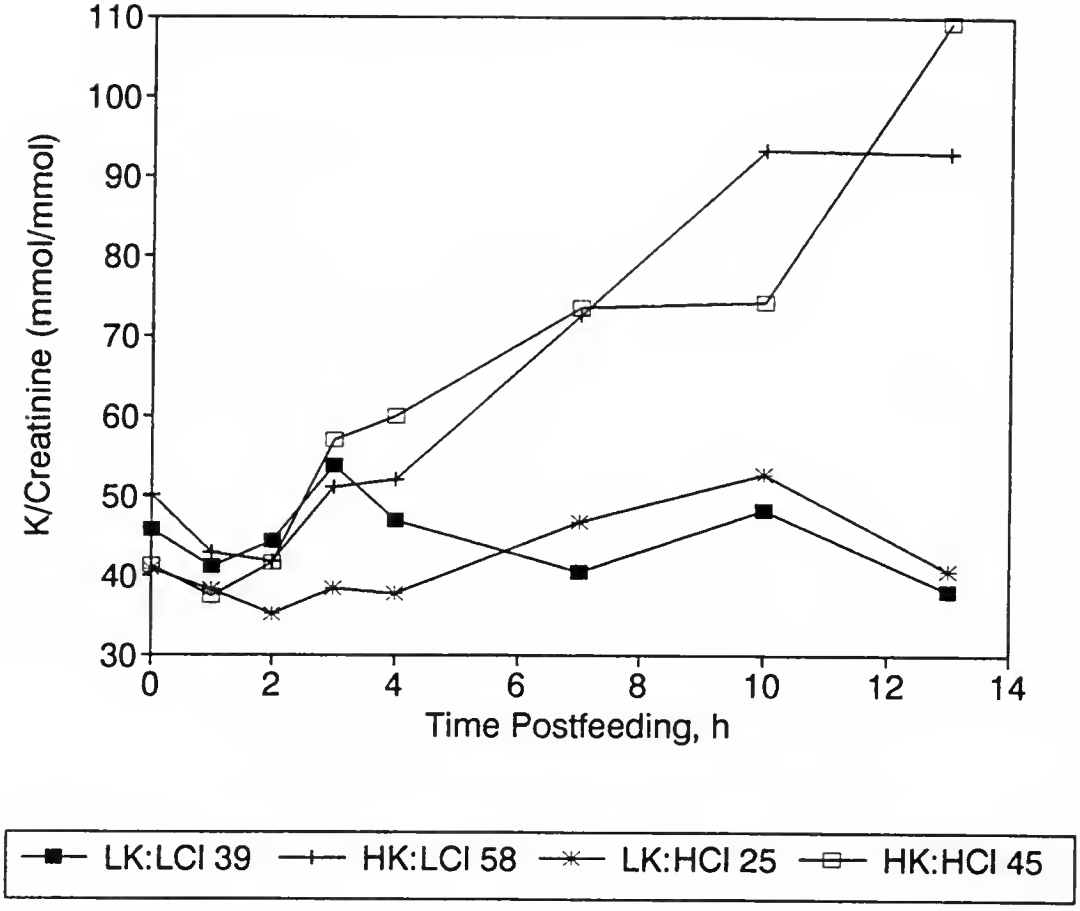


Figure 6-4. Least squares means of urine K/creatinine ratio during d 1. LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl). Values for CAD are next to treatment designations in figure legend. Standard error of the mean = 7.22 mmol/mmol.

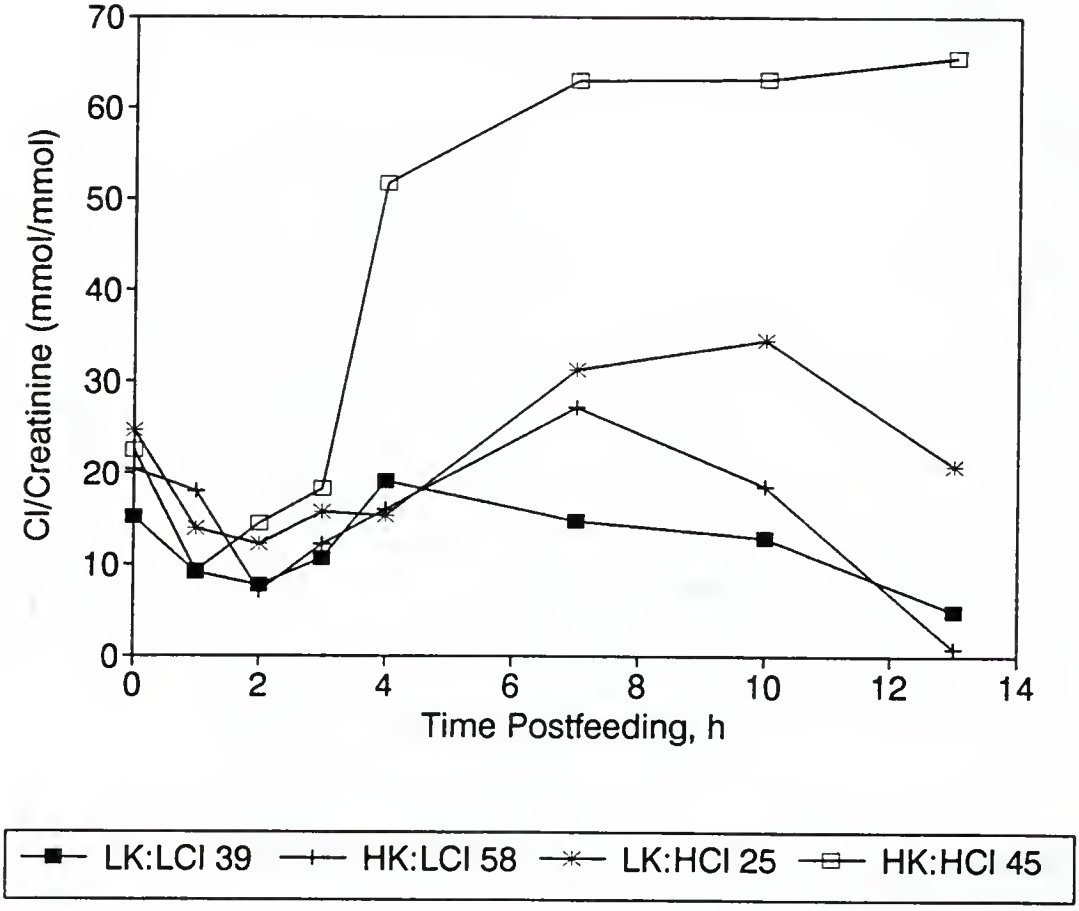


Figure 6-5. Least squares means of urine Cl/creatinine ratio during d 1. LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl). Values for CAD are next to treatment designations in figure legend. Standard error of the mean = 5.67 mmol/mmol.

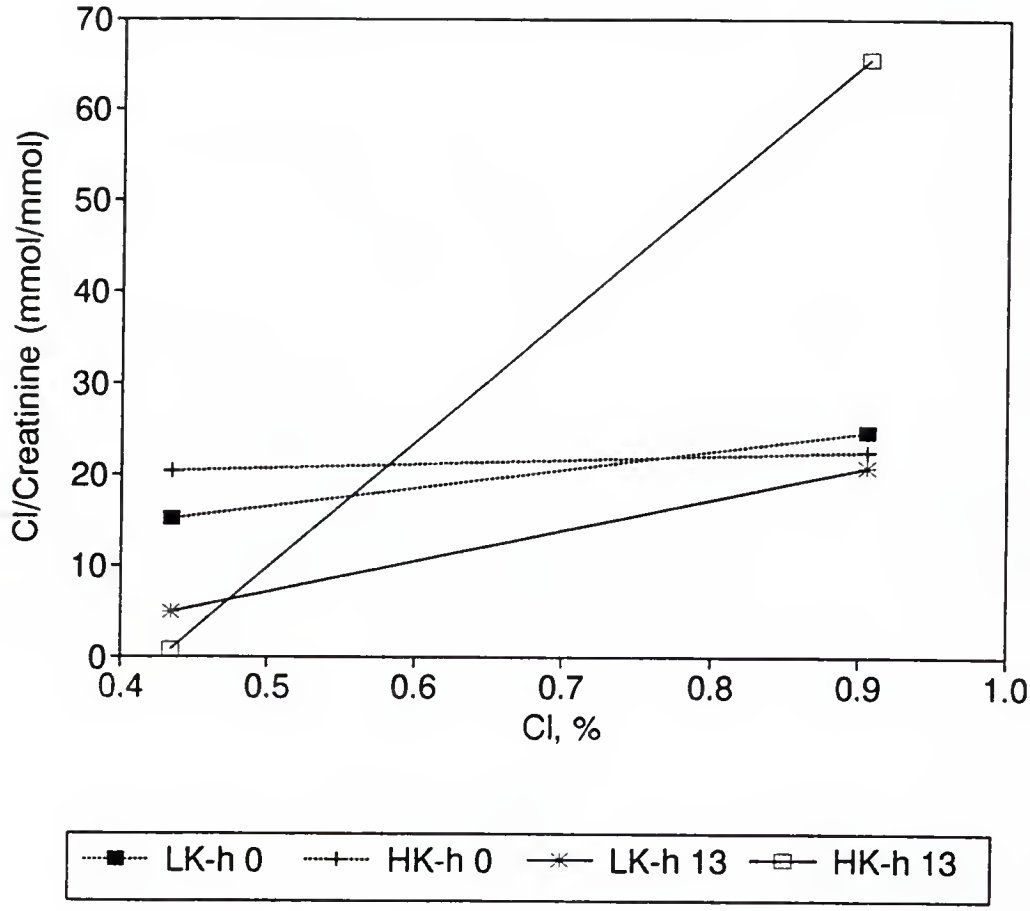


Figure 6-6. Least squares means of urine Cl/creatinine ratio at h 0 and h 13 on d 1. LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl). Values for CAD are next to treatment designations in figure legend. Standard error of the mean = 5.67 mmol/mmol.

postfeeding the interaction between K and Cl was more obvious (solid lines). At 13 h after feeding, increasing dietary Cl (from .43 to .91%) increased urinary Cl/creatinine about 3 times greater in high K diets than in low K diets. In Tucker et al. (1988b), cows fed diets with .86% Cl were able to eliminate excess Cl by 4 h postfeeding. However, the high Cl (.86% Cl) diets in that study had greater Na (1.1 vs. .54 and .55% Na) and CAD (+56 vs. +25 and +45 CAD) than diets in the present study. In the present study, a lack of dietary K reduced normal concentrations of urinary Cl/creatinine in cows fed high Cl. Concentrating Cl in urine apparently required K as a counter ion. Instead of being excreted, excess Cl from the LK:HCl diet was retained in plasma (Table 6-3) and milk (Table 6-8). As a strong ion, Cl likely was responsible for accompanying changes in blood-acid base status (Stewart, 1981). To maintain electrical balance, H^+ and Ca^{++} concentrations in blood were elevated. Calcium ions originated from either dissociation of Ca bound to plasma protein (Moore, 1970), increased Ca absorption (Fredeen et al., 1988), or Ca mobilized from bone (Block, 1984). Mobilization of Ca from bone also releases $CO_3^=$ ions (Stacy and Wilson, 1970) which can buffer H^+ ions. This may be the reason no effect of dietary treatment on blood HCO_3^- was observed in this experiment. Increased urine Na/creatinine also may have been compensating for lack of K cations for excretion with Cl. The bovine kidney can synthesize and secrete NH_4^+ as an additional means to eliminate H^+. However, renal mechanisms are the most sluggish of the acid-base regulatory systems (Hilwig, 1976). The large variance in NH_4^+ coupled with its delayed synthesis may explain why differences in

urinary NH_4^+ were not detected. Escobosa et al. (1984) and Tucker et al. (1988b) did not measure urinary NH_4^+.

Effects of dietary CAD on urinary Cl/creatinine did not accompany the Cl and K x Cl effects ($P > .1$). However, urinary Ca/creatinine decreased with increasing dietary CAD (linear CAD effect, $P < .05$) Table 6-4). Urinary Ca/creatinine tended to decrease with increasing dietary K ($P < .1$). Increasing dietary CAD for dry cows (Wang and Beede, 1992; Oetzel, 1988; and Block, 1984) consistently resulted in decreased urinary Ca, indicating that the mechanisms responsible are similar in dry and lactating cattle. No effects of treatment on urinary Mg/creatinine were observed ($P > .1$).

Phase II--Daily Sampling

Blood acid-base status and mineral metabolism. Blood responses at 4 h postfeeding during Phase II (d 1 through 7) were influenced primarily by Cl and the linear effect of CAD (Table 6-5). Blood H^+ ($P < .05$), ionized Ca ($P < .01$), and plasma Cl ($P < .01$), increased, and plasma protein tended to decrease ($P < .1$) with increasing dietary Cl. Blood H^+ ($P < .05$), ionized Ca ($P < .01$), and plasma Cl ($P < .05$) increased, and plasma HCO_3^- and plasma protein tended to decrease with decreasing CAD. Escobosa et al. (1984) also observed increased blood [H^+] with a high Cl diet (1.65% Cl).

Plasma Cl tended to increase during each period (linear d effect, $P < .1$), but time did not interact ($P > .1$) with treatment responses. Tucker et al. (1988b) also sampled cows in hourly and daily intervals. As in their hourly phase, they did not observe increased plasma Cl with

TABLE 6-5. Effects of dietary K, Cl and cation-anion difference (CAD) on blood variables not affected by dietary treatment by d interactions.[1]

Variable	Dietary Treatments[2,3] LK:LCl CAD 39	HK:LCl CAD 58	LK:HCl CAD 25	HK:HCl CAD 45	SEM[4]
H+, neq/L[a,b]	34.06	34.03	35.84	34.61	0.51
pH[5]	7.468	7.468	7.446	7.461	
iCa, meq/L[c,d]	2.25	2.22	2.32	2.29	0.03
Plasma Na, meq/L	142.98	143.68	143.07	143.75	0.72
Plamsa K, meq/L	3.90	4.09	3.97	4.22	0.15
Plasma Cl, meq/L[b,c]	102.96	102.91	105.23	104.43	.66
Plasma HCO_3^-, meq/L[e]	24.79	25.55	24.09	25.07	0.62
Red blood cell K, meq/L	18.38	18.57	18.98	18.64	0.45
Hematocrit, %	28.74	29.15	28.70	29.74	0.57
Plasma protein, mg/dl[e,f]	7.61	7.70	7.37	7.47	0.13

[1]Least squares means pooled across Phase II sampling times (d 1 through 7, 4 h postfeeding).

[2]LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl).

[3]CAD = meq (Na + K - Cl)/100g diet DM.

[4]Standard error of the mean.

[5]Calculated from H^+ concentration; not evaluated statistically.

[a]Difference due to dietary Cl ($P < .05$).

[b]Difference due to linear effect of dietary CAD ($P < .05$.)

[c]Difference due to dietary Cl ($P < .01$).

[d]Difference due to linear effect of dietary CAD ($P < .01$).

[e]Difference due to linear effect of CAD ($P < .1$).

[f]Difference due to dietary Cl ($P < .1$).

.86% vs. .34% Cl after 14 d of feeding higher Cl. These contrasting results between their study and the present one likely were due to much greater Na and CAD in their high Cl diets than in the high Cl diets in the present study.

No effects of dietary K, Cl or CAD on plasma Na, plasma K, red blood cell K or blood hematocrit were observed ($P > .1$) in Phase II. Changes in plasma K and red blood cell K must have been very rapid because they occurred during Phase I but not during Phase II. This may explain why increased plasma K was not observed in a previous experiment conducted here (Sanchez et al., 1990a) (chapter 3) when samples were taken four weeks after introducing new diets.

Urinary cation and anion concentrations. As in Phase I, urinary H^+ concentrations were higher in cows fed low CAD diets during Phase II (linear effect of CAD, $P < .05$; Table 6-6). Again, the LK:HCl diet was numerically most influential, but was not statistically compared with other diets. Tucker et al. (1988b) also noted increased urine H^+ when low CAD diets were fed for 14 d. Urine [H^+] was increased by feeding high Cl in another study with lactating dairy cows (Escobosa et al., 1984). In the current study, treatment effects on urinary mineral concentrations during Phase II were similar to treatment effects during Phase I. The major difference was that K x Cl and time x K x Cl interactions occurred more often in the Phase II.

Urinary Na/creatinine was influenced by K x Cl, and d x K x Cl interactions ($P < .05$). Overall, the K x Cl interaction indicated that urinary Na/creatinine response to dietary K depended upon dietary Cl

TABLE 6-6. Effects of dietary K, Cl and cation-anion difference (CAD) on urine variables not affected by dietary treatment by d interactions.[1]

Variable	Dietary Treatments[2,3] LK:LCl CAD 39	HK:LCl CAD 58	LK:HCl CAD 25	HK:HCl CAD 45	SEM[4]
H+, neq/L[a]	6.56	3.98	32.31	6.83	8.15
pH[5]	8.183	8.400	7.491	8.165	
$NH4_4^+$/Creatinine, mmol/mmol	.59	.73	.39	.48	.44
K/Creatinine, mmol/mmol[a,b]	36.58	77.11	33.86	70.87	6.86
Cl/Creatinine, mmol/mmol[c,d,e,f]	9.36	5.70	19.05	39.43	5.99
Ca/Creatinine, mmol/mmol[e,g]	.213	.155	.433	.148	.101
Mg/Creatinine, mmol/mmol	7.63	8.37	8.24	6.64	.873

[1]Least squares means pooled across Phase II sampling times (d 1 through 7, 4 h postfeeding).

[2]LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl).

[3]CAD = meq (Na + K - Cl)/100 g diet DM.

[4]Standard error of the mean.

[5]Calculated from H^+ concentration; not evaluated statistically.

[a]Difference due to linear effect of CAD ($P < .01$).

[b]Difference due to dietary K ($P < .01$).

[c]Difference due to dietary Cl ($P < .05$).

[d]Difference due to K x Cl interaction ($P < .05$).

[e]Difference due to linear effect of CAD ($P < .05$).

[f]Difference due to quadratic effect of CAD ($P < .05$).

[g]Difference due to dietary K ($P < .1$).

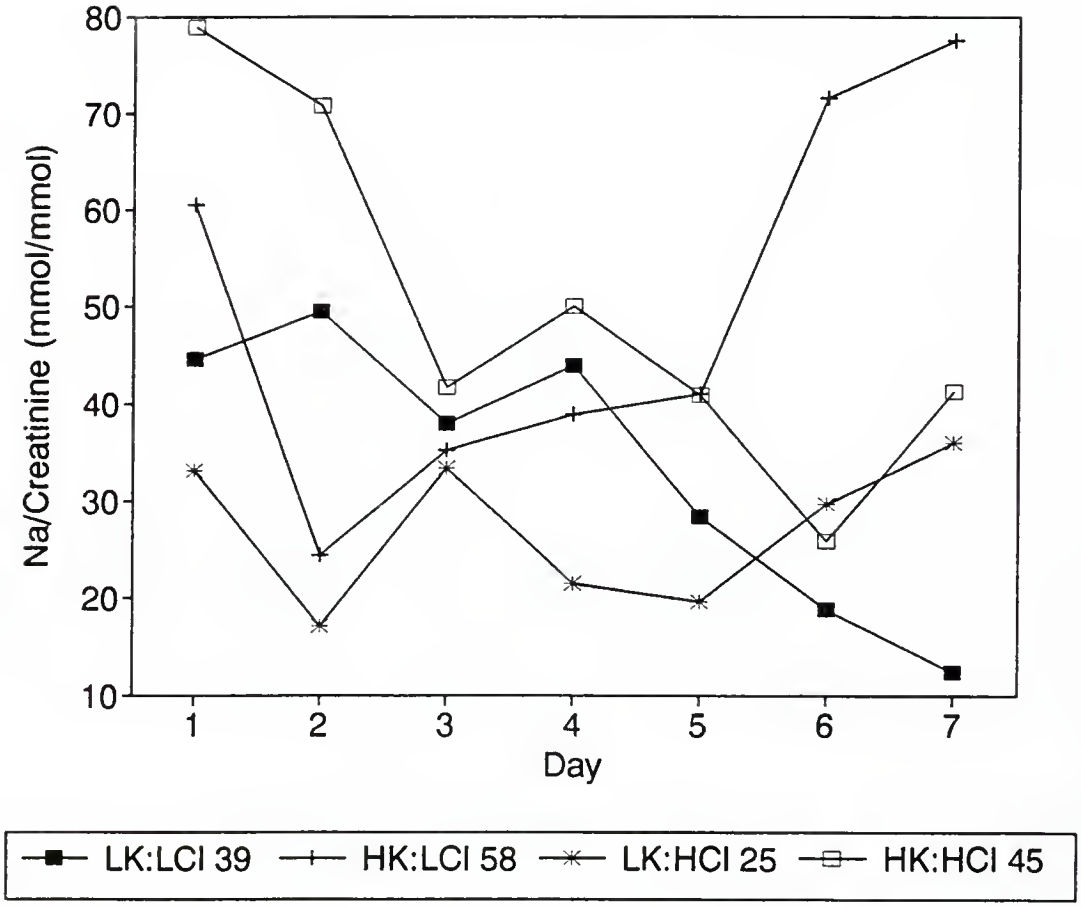


Figure 6-7. Least squares means of urine Na/creatinine ratio during d 1 through 7. LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl). Values for CAD are next to treatment designations in figure legend. Standard error of the mean = 10.03 mmol/mmol.

concentrations (Figure 6-7). This effect was difficult to interpret, particularly when combined with time (K x Cl x d effect). During d 1, urine from cows fed LK:HCl had 42% less Na/creatinine than from cows fed HK:HCl (33 vs. 79 mmol/mmol). By d 7 this difference disappeared and the greatest concentration of urinary Na/creatinine was from cows fed HK:LCl. Urinary Na/creatinine also responded curvilinearly to CAD (quadratic effect of CAD, $P < .05$) and depended upon d (CAD x CAD x d interaction, $P < .05$) (Figures not shown).

Urine K/creatinine increased with increasing dietary K and CAD ($P < .01$). Treatment responses did not differ over d (Table 6-6; $P > .1$).

Urinary Cl/creatinine responses to Cl and K x Cl observed in Phase I continued through Phase II. Increasing dietary Cl increased urine Cl/creatinine, but with high K diets the increase was much greater. With low K diets, increasing dietary Cl from .43 to .91% resulted in a 2-fold increase in urine Cl/creatinine (19.05 vs. 9.36 mmol/mmol). In contrast, with high K diets this same increase in dietary Cl increased urine Cl/creatinine by 7-fold (39.03 vs. 5.70 mmol/mmol) (Table 6-6; Figure 6-8). Eliminating large amounts of dietary Cl fed over 7 d presumably continued to require sufficient dietary K. Although in apparent contradiction, findings of Tucker et al. (1988b), in fact, support this supposition. They reported increased urine Cl/creatinine with higher Cl diets (.86 vs. .34% Cl). But there were four differences in their study compared to this one. The high Cl diets fed in their study had: (1) greater Na (1.01% vs. .54%); (2) greater CAD (+56 vs. +25); (3) slightly greater K (1.16% vs. 1.07%); and (4) slightly greater Ca (.90 vs. .78%) concentrations. It is likely that cows in their study

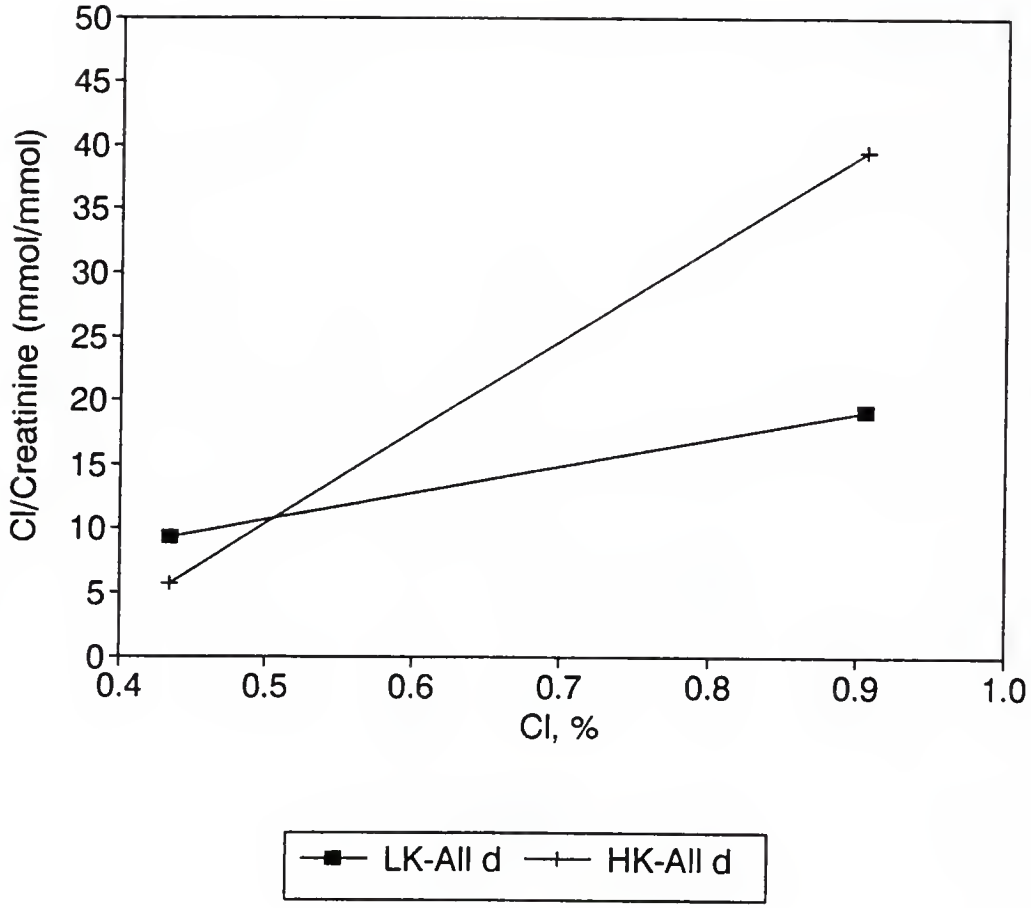


Figure 6-8. Least squares means of urine Cl/creatinine ratio during d 1 through 7. LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl). Values for CAD are next to treatment designations in figure legend. Standard error of the mean = 5.99 mmol/mmol.

were able to eliminate excess Cl because they consumed diets with greater concentrations of fixed cations. As a result, plasma Cl was not elevated with higher dietary Cl in their study. Because neither study included mineral balance measurements, comparing total mineral excretion is not possible.

In the present study, urinary Cl/creatinine responded curvilinearly to CAD (quadratic CAD effect, $P < .05$). Urinary Cl/creatinine response to K, Cl, K x Cl and CAD did not differ over time.

Increasing dietary K tended to decrease urinary Ca/creatinine concentrations ($P < .1$). Increasing dietary CAD decreased urinary Ca/creatinine ($P < .05$). These effects were consistent with those in Phase I. Tucker et al. (1988b) also observed an increase in urinary Ca/creatinine with high dietary Cl when fed for 14 d. As in Phase I, no effects of treatment on NH_4^+/creatinine and Mg/creatinine were observed in Phase II of this experiment. Urinary Mg/creatinine also did not differ between treatments in Tucker et al. (1988b); urinary NH_4^+ was not measured in their study.

Fecal Composition and Apparent Mineral Digestibility. Fecal samples were collected to determine if fecal composition and mineral and DM digestibilities responded differently to treatments (Table 6-7). High dietary K increased apparent K digestibility by 5 percentage units (90.2 vs. 85.9, averaged across Cl concentrations; $P < .01$). Similarly, high dietary Cl increased Cl digestibility by 25 percentage units (82.5 vs. 66.1, averaged across K concentrations, $P < .01$). Cation-anion difference also affected K and Cl digestibility. Increasing dietary

TABLE 6-7. Effects of dietary K, Cl and cation-anion difference (CAD) on fecal composition and apparent mineral digestibilities not affected by dietary treatment by d interactions.[1]

	Dietary Treatments[2,3]				
Variable	LK:LCl CAD 39	HK:LCl CAD 58	LK:HCl CAD 25	HK:HCl CAD 45	SEM[4]
Fecal dry matter, %	18.90	19.19	18.21	18.14	.71
Fecal H^+	965.57	1096.76	1141.72	902.81	261.87
Fecal pH[5]	6.015	5.960	5.942	6.044	
Dry matter digestibility, %	59.84	56.85	58.79	61.07	4.49
Na digestibility, %	83.83	86.33	83.97	87.45	2.21
K digestibility, %[a,b]	85.68	89.58	86.17	90.81	1.54
Cl digestibility, %[c,d]	65.31	66.81	81.54	83.39	3.58
Ca digestibility, %	-6.314	-1.25	.54	.89	11.39
Mg digestibility, %	9.18	6.52	11.69	10.81	9.15

[1]Least squares means pooled across Phase II sampling times (d 1 through 7, pooled a.m. and p.m. fecal samples).

[2]LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl).

[3]CAD = meq (Na + K - Cl)/100 g diet DM.

[4]Standard error of the mean.

[5]Calculated from H^+ concentration; not evaluated statistically.

[a]Difference due to dietary K ($P < .01$).

[b]Difference due to linear effect of dietary CAD ($P < .05$).

[c]Difference due to dietary Cl ($P < .01$).

[d]Difference due to linear effect of dietary CAD ($P < .01$).

CAD increased K digestibility but reduced Cl digestibility. When DMI was fit as a continuous independent variable (covariate), K and Cl digestibility remained significantly greater ($P < .01$) with high dietary K and Cl, respectively. However, changes in DMI were partially responsible for response to dietary CAD. With DMI included as a covariate, increasing dietary CAD only tended ($P < .1$) to increase K digestibility and did not ($P > .1$) increase Cl digestibility. A significant association between dietary K (from .4 to 2.8% diet DM) and digestibility was not observed by Paquay et al. (1969b). However, in their report K digestibility increased hyperbolically; increasing rapidly when dietary K increased from .4 to 1% but plateaued off after 1% K. When digestible K was plotted against dietary K content, a significant linear regression ($r = .961$; $P < .01$) was observed (Paquay et al., 1969b). From these results coupled with the influence of DMI on fecal K excretion also observed in their study (Paquay et al., 1969b), they concluded that reduced apparent K digestibility with low K was due to increased endogenous K in the feces. In the current experiment, fecal DM and $[H^+]$, and Na, Ca, Mg and DM digestibilities were not affected by treatment, time or treatment x time interactions ($P > .1$).

Lactational Performance. Lactational performance generally was unaffected in the short time periods used in this experiment (Table 6-8). Milk composition, however, was altered by dietary treatment. Milk Cl tended to increase with high dietary Cl ($P < .1$) compared with low Cl. Milk H^+ increased with high Cl ($P < .01$) and high K compared to low Cl and K, respectively. The increased H^+ in milk suggests that increased anionic Cl charges in milk of cows fed high dietary Cl were

TABLE 6-8. Effects of dietary K, Cl and cation-anion difference (CAD) on dry matter intake (DMI), milk yield and composition variables not affected by dietary treatment by d interactions.[1]

Variable	Dietary Treatments[2,3] LK:LCl CAD 39	HK:LCl CAD 58	LK:HCl CAD 25	HK:HCl CAD 45	SEM[4]
DMI, kg/d	16.41	16.70	16.57	16.28	.53
Milk yield, kg/d	19.75	20.29	20.17	19.88	.35
Milk H^+, neq/L[a,b]	223.60	241.08	246.34	266.67	7.34
Milk pH[5]	6.651	6.618	6.608	6.574	
Milk fat, %	3.58	3.94	3.85	3.76	.18
Milk protein, %	3.01	3.00	3.12	3.06	.06
Milk lactose, %	4.82	4.80	4.88	4.84	.09
Milk solids, %	8.58	8.56	8.75	8.66	.19
Milk Na, meq/L	16.20	16.37	17.26	16.92	.88
Milk K, meq/L	33.96	34.02	34.88	34.79	1.46
Milk Cl, meq/L[c]	31.20	31.66	34.28	33.44	1.51
Milk Ca, meq/L	43.43	43.95	44.47	43.92	2.03
Milk Mg, meq/L	7.03	7.25	7.30	7.62	.47

[1]Least squares means pooled across Phase II sampling times (d 1 through 7, total DMI and pooled a.m. and p.m. milk samples).

[2]LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl).

[3]CAD = meq (Na + K - Cl)/100 g diet DM.

[4]Standard error of the mean.

[5]Calculated from H^+ concentration; not evaluated statistically.

[a]Difference due to dietary K ($P < .01$).

[b]Difference due to dietary Cl ($P < .01$).

[c]Difference due to dietary Cl ($P < .1$).

countered with H^+ ions (Table 6-8). Significant main effects of dietary Cl and K on milk Cl and H^+ composition did not translate into significant effects of dietary CAD on these variables. Other performance (DMI and MY) and milk composition variables were unaffected by K, Cl, K x Cl or CAD.

No effects of dietary treatments on BW gain or respiration rate were observed. Means for LK:LCl, HK:LCl, LK:HCl, and HK:HCl were 11.4, 56; 2.9, 52; 7.6, 51; and -5.3, 51, for BW gain (kg/week, SEM = 6.9) and respiration rate (breaths/min, SEM = 3.9), respectively.

Rectal temperature responded curvilinearly through time (quadratic d effect, $P < .05$; Figure 6-9) and tended to decrease with high dietary Cl ($P < .1$). When DMI was included in statistical models as a continuous independent variable (covariate), temperature was still lower ($P < .05$) with high vs. low dietary Cl, indicating that the effect of dietary Cl on rectal temperature was independent of DMI. Coppock et al. (1982) and Escobosa et al. (1984) also observed reduced body temperature with high dietary Cl. They suggested that reduced intakes on the high Cl diets were responsible for reduced rectal temperatures, but did not include DMI as a covariate in rectal temperature ANOVA models. Cation-anion difference did not influence body temperature ($P > .1$).

Discussion

In the short time periods used in this study, urinary Cl/creatinine ratio was one of the only response variables affected by dietary K x Cl interaction. Nevertheless, the nature of this response provided a rationale for lactational responses to dietary K x Cl

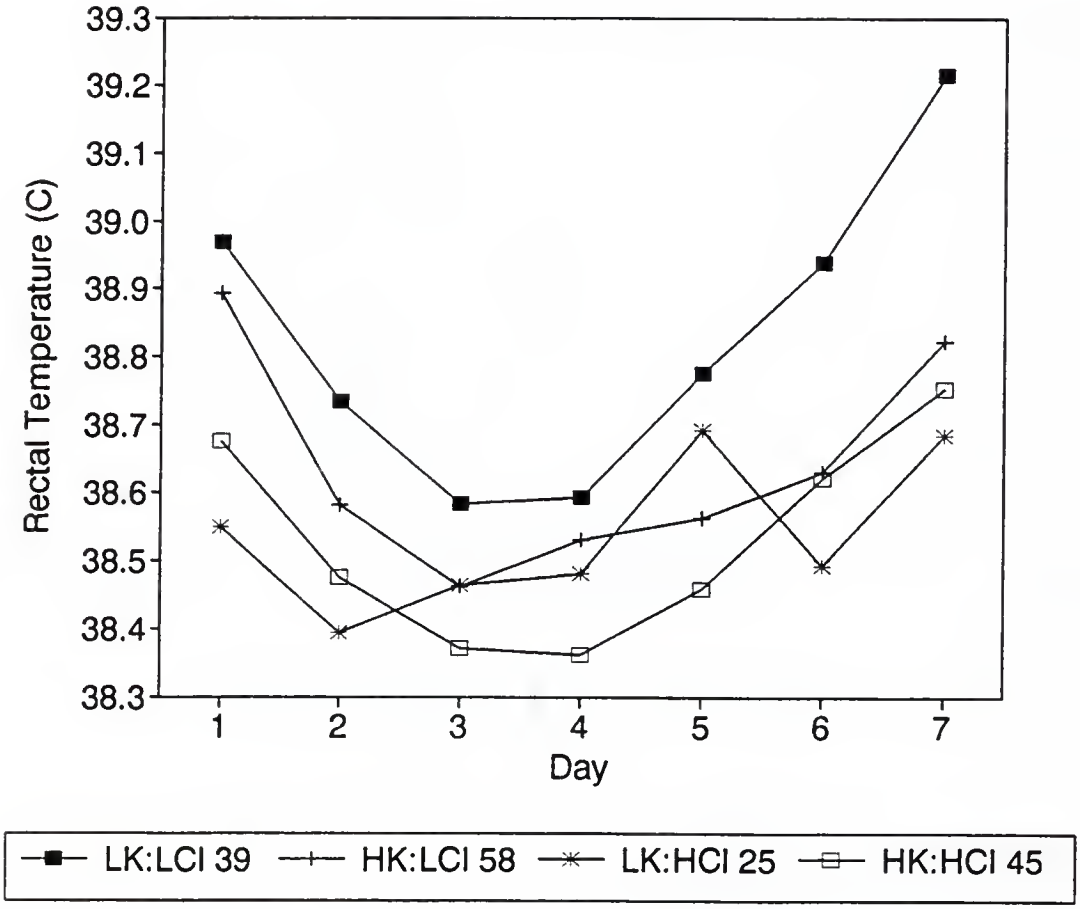


Figure 6-9. Least squares means of rectal temperature during d 1 through 7. LK:LCl = Low K, Low Cl (1.11% K, .43% Cl); HK:LCl = (High K, Low Cl (1.72% K, .44% Cl); LK:HCl = Low K, High Cl (1.07% K, .91% Cl); HK:HCl = High K, High Cl (1.80% K, .90% Cl). Values for CAD are next to treatment designations in figure legend. Standard error of the mean = .11 ° C.

interaction observed previously (Sanchez et al., 1990a; Sanchez et al., 1991) (chapter 3 and 5 in this dissertation). Increases in dietary Cl increased plasma Cl, milk Cl and urine Cl. Yet, with a diet high in Cl but low in K, urinary excretion of Cl was hindered. This resulted in an increase in plasma and milk Cl. Hydrogen and Ca^{++} ions counterbalanced excess Cl in plasma and H^{+} ions countered excess Cl in milk. Blood ionized Ca and urine Ca increased with this diet also. This sequence of events most likely occurs because urinary excretion of excess dietary Cl requires adequate dietary K. A diet with high Cl, but low K (i.e., 1.07% K, .91% Cl) did not contain sufficient cations to accompany excess Cl anions into the urine and thus Cl was retained.

Another purpose of this experiment was to separate individual effects of dietary K and Cl from their combined effects. This was done by evaluating the effects of dietary K, Cl, and K x Cl in one model and CAD in another. Most of the responses that were affected by either K or Cl also were affected by CAD. Therefore the current findings are in agreement with Tucker and Hogue (1990), who determined that dietary CAD was a useful indicator of the physiological effects of monovalent macrominerals on lactating dairy cattle. However, because concentrations of the individual ions are confounded with dietary CAD, conclusions about the effects of CAD must be made with caution. Dietary CAD is a useful expression to predict the physiological effects of K and Cl, but it is the ions themselves that have the physiological effect.

In this study, although CAD had significant effects on most responses, most of the response variables were influenced by the individual effects of either dietary K or Cl. Only two responses were

influenced by diet concentrations of both minerals: blood H^+ (in Phase I) and milk H^+ (Phase II). All other responses were influenced by just one dietary mineral. Whether or not this was a result of the short time periods used in this experiment will require further study. Using longer time periods (3 wk), Tucker and Hogue (1990) suggested that CAD is a more important determinant of dietary impact on systemic acid-base status than actual dietary concentrations of Na, K and Cl. But even if CAD effects are different over longer periods, it should prove valuable to consider both the individual ions and CAD at the same time. Dietary CAD may be an important determinant of chronic effects of dietary K and Cl. Because CAD combines effects of both elements, it is useful in studying responses that are affected by interactions between dietary K and Cl. However, the individual ions evaluated separately will facilitate further definition of their individual physiological effects, particularly in the acute postprandial interval.

CHAPTER 7
SUMMARY AND RECOMMENDATIONS FOR FUTURE RESEARCH

Overall objectives were to determine the influence of dietary Na, K, and Cl on acid-base status and lactational performance of dairy cattle. Determining the influence of dietary Na, K and Cl interrelationships, particularly the three-way dietary CAD interrelationship, on lactational and physiological responses was fundamental.

Each of the projects in the dissertation were conducted sequentially, with the findings of each applied to the next. As this information was accumulated it was used to design of subsequent projects.

Because specific interrelationships among dietary Na, K and Cl were not understood at the onset of this research, experiment one was conducted. In this study objectives were three-fold: (1) to study productive and physiological responses to varying concentrations of dietary Na, K and Cl; (2) to determine if optimal dietary concentrations of each were interrelated; and (3) to determine if dietary CAD was related to physiological and lactational responses. Numerous interrelationships were discovered for several acid-base status, mineral metabolism and lactational response variables. The optimum CAD range was narrowed.

It was unknown if responses presumably due to concentrations of Na, K or Cl were confounded with dietary source of Na, K and Cl. Therefore, objectives of experiment two were to investigate responses due to common commercial sources of Na, K and Cl. Diets with mixtures of $NaHCO_3$, NaCl and KCl were formulated and fed to dairy cattle to measure acid-base status, mineral metabolism and lactational responses. Because the differing concentrations of mineral sources altered dietary CAD, an additional objective was to further explore response relationships to CAD. Results from that experiment indicated that effects of individual ions probably were not influenced by mineral source. Additional information on optimal CAD concentration was obtained.

Although a total of 84 cows were used in the first two studies, it became apparent that to study all potential interrelationships among Na, K and Cl over a wide range of CAD, many more experiments would be required. Fortunately several years of data from previous macromineral nutrition experiments conducted at the University of Florida were available. Data from the first two experiments in this dissertation were combined with eight other data sets and an analysis was performed on the entire data base. Concentrations of Na, K and Cl, as well as several other macrominerals, ranged from below minimum recommendations to concentrations considerably above recommendations. Dietary CAD also encompassed a large range. In general, results from that project were helpful to describe the nature of several macromineral interrelationships and to pinpoint the optimal CAD for lactating dairy cattle. Consistent interrelationships between Na and K, Na and Ca, K

and Ca, and K and Cl were found. Performance was optimal at .58% Na, .40% Mg and +38 CAD.

In two of the projects, an interaction between dietary K and Cl had a large influence on economically important response variables. There was a consistent effect, but the physiological mechanism was not understood. Therefore, a final experiment was conducted to characterize physiological responses of lactating dairy cattle to dietary K x Cl interaction, and to determine if responses were related to CAD. Results obtained were sufficient to propose a physiological mechanism which could explain how and why an interrelationship between dietary K and Cl existed. A diet with high Cl but low K did not contain sufficient cation to accompany excess Cl into urine. Chloride was retained and subclinical metabolic hyperchloremic acidosis developed.

Results obtained should resolve some of the discrepancies reported on optimal dietary concentrations of Na, K and Cl for lactating dairy cattle. The need to consider dietary macromineral interrelationships and CAD was established.

Results have generated ideas for future research. In the opinion of the author, future research should address the following.

1. Specific macromineral interrelationships. Interrelationships that had consistent effects for several lactational response variables included Na x K, Na x Ca, K x Ca, and K x Cl. The final project addressed the K x Cl interaction, but little information exists on the others. Additional research should address each of these. The contention that Na and K spared one another will require

substantiation. Intensive, repeated sampling experiments like that conducted in chapter six will be useful to confirm or reject the significance of this and the other interrelationships. The K x Cl interrelationship also needs further characterization. From the results of chapter six, cows appear to tolerate high K, low Cl diets much more so than low K, high Cl diets. Therefore, an additional experiment should be conducted with low K, high Cl diets. Total collection of urine should help to determine if low dietary K truly did impede urinary excretion of excess dietary Cl as was hypothesized.

The milk protein depression due to the K x Cl interrelationships observed in experiment one (chapter three) was very dramatic and should be studied further. Predicted milk protein percentage increased from about 2.7 to 3.0 percentage units (11% increase) when dietary K increased from .9 to 1.9% and dietary Cl was increased from .3 to 1.3% simultaneously (Figure 3-8). If either mineral was increased without increasing the other, the response was negative. Because milk protein concentration is critical to cheese yield, several milk cooperatives have begun paying premiums based on milk protein concentration. Any impact K x Cl interrelationships have on milk protein concentration thus could impact the profitability of the dairy farmer significantly. The lack of effect of K x Cl on milk protein concentration in the final experiment (chapter six) should not be used to rule out the possibility that dietary K and Cl concentrations affect milk protein. Others have reported effects of these minerals on milk

protein concentration (see chapter 3 and 4 for discussion). The lack of effect may have been due to the relatively short experimental period used in the last experiment. Milk protein synthetic mechanisms involve regulated sequences of amino acid uptake by the gland, transcription of milk-protein genes, translation of milk protein messenger RNA, and co-transnational and post-transnational modification of pre-secretory milk proteins (Dils, 1989). Changes in these mechanisms potentially mediated by K x Cl interrelationships likely required longer than the 7-d experimental periods used in chapter six, but were likely in effect after the 4-wk periods used in chapter three. A longer and more direct evaluation of protein and amino acid metabolism should be included in any future study of the K x Cl interrelationship.

2. Prediction models. If the significance of the K x Cl interrelationship and other mineral interrelationships are confirmed, coefficients should be incorporated into prediction models. However, even before the significance of two-way macromineral interrelationships are confirmed, current prediction models used by the dairy industry should incorporate dietary CAD model coefficients. Several prediction models have been published that relate feed intake and milk production to dietary nutrient concentrations. These types of prediction models are an integral component of dairy farm enterprise models. Accurate prediction of inputs and outputs dictate the usefulness of dairy farm enterprise models and thus profitability of the dairy. Currently none of the models used contain macromineral variables. In the opinion of the

author, macromineral variables such as dietary CAD should improve the usefulness of current models.

3. Variables correlated with blood acid-base status. It was hypothesized and confirmed that the influence of macromineral interrelationships were via alterations in acid-base status. However, acid-base measurements are expensive and difficult to collect. Blood samples need to be chilled, kept anaerobic, and processed very rapidly after collection. Further, these samples can only be analyzed using expensive blood-gas analysis equipment that is often cost prohibitive for most animal nutrition laboratories. Identifying variables that are correlated to blood gas measures (blood pH, pCO_2, HCO_3^-) should be of value. Ionized Ca is a good candidate, but it also is an expensive and infirm analyte. Plasma and milk Cl, which can be assayed from frozen aerobic samples, appear to be correlated significantly with metabolic acidosis. A study should be conducted to confirm this and to identify other variables potentially correlated with acid-base status. Statistical procedures such as the meta-analysis procedure used by Oetzel (1991) in the identification of nutritional risk factors associated with milk fever may prove useful in identifying these variables if they exist.

4. Practical importance. Although there appears to be important effects due to macromineral interrelationships, one could challenge the practical significance. For example, in experiment one and three (chapter 3 and 6), $CaCl_2$ was used as a source of Cl. There have been reports that feedstuffs vary considerably in Cl

concentrations. There also are several methods currently used to analyze Cl in feedstuffs. This indicates that Cl concentrations fed to lactating dairy cattle may vary widely. Whether or not organic sources of Cl cause the same detrimental effects that $CaCl_2$ did will require further study. Further, it is not known how much Cl concentrations vary in commercial diets (that do not include $CaCl_2$) and if variable concentrations are detrimental. Commercial diets should be surveyed to determine average concentrations of Cl (and other macrominerals) to evaluate the practical significance of potential Cl x macromineral interrelationships.

The author plans to publish results presented in this dissertation and is hopeful that they will be used by nutritionists that currently feed commercial dairy cows. The author recommends that the optimal CAD concentrations determined in the various projects be used. When diets contain excessive mineral anions relative to mineral cations and CAD values are below +20, increases in lactational performance can be attained simply by elevating CAD to values within the range of +20 to +50. However, potential limitations of use of CAD in diet formulation should be noted.

Most important, CAD calculations will not be useful in situations where diets contain very high or very low concentrations of both mineral anions and mineral cations (i.e., when Na, K and Cl are present in deficient or toxic concentrations). A diet could contain toxic concentrations of Na and Cl, but contain what might appear to be optimal

CAD. For example, NRC (1980) indicates that 4% NaCl is the maximum tolerable dose for lactating dairy cattle. Cows fed a diet a toxic level of NaCl (i.e., 5% NaCl) will undoubtedly yield less milk than cows fed a diet with adequate NaCl (i.e., .5% NaCl) even though CAD would be the same in both diets (NaCl does not alter CAD). Dietary toxicities would need to be corrected before CAD would be a useful measure of lactational performance. The same case can be made for diet deficiencies.

There is one other potential limitation to the CAD expression. Inherent in the CAD expression may be an inaccurate assumption. While the CAD concept correctly distinguishes cations from anions, it implies that their individual effects are identical. In other words, all ions are weighted the same in the expression. The validity of this assumption has not been tested. It is very likely that one ion is more influential on a specific response than the other ions. Potential two-way interrelationships are probable and could prove to be very important.

Although there are potential limitations which need to be studied further, results from research reported in this dissertation are directly applicable to the dairy industry and should provide economic returns. We now can recommend optimal CAD concentrations and have information on the influence of specific macromineral interrelationships. Nutrition consultants should use this information to formulate optimum concentration of minerals in diets that meet the needs of todays high producing dairy cows. Nutrition researchers should use this information to design future studies that further address

specific macromineral interrelationships. And dairy scientists should use this information to improve dairy farm enterprise models.

Feeding todays lactating dairy cow is a still a tremendous challenge. But using the information gathered in this dissertation should improve the efficiency of converting low quality feed into a high quality human food: milk.

APPENDIX A
STATISTICAL TABLES FOR CHAPTER 3

TABLE A-1. Parameter estimates, R^2, response mean, surface type and factor critical value[1], of full quadratic models for dry matter intake (DMI,kg/d), milk yield (MY,kg/d), 3.5% FCM (kg/d), milk fat (MF, %), milk protein (MP,%) and body weight change (BWG,kg/d) for chapter 3.

Parameter Estimates	R^2	Response Mean	Surface Type	Factor Critical Values (%) Na	K	Cl
DMI=18.04 +3.99Na +5.47K -2.04Cl +.07Na^2 -1.59K^2 -3.50Cl^2 -5.30Na*K +6.26Na*Cl +2.92K*Cl	.73	22.49	Saddle point	.44	1.75	.84
MY=20.53 -1.72Na +1.55K +.27Cl +3.71Na^2 -.92K^2 -4.89Cl^2 -2.88Na*K +3.90Na*Cl +3.92K*Cl	.77	21.75	Saddle point	.63	OHER[2]	OHER[2]
3.5% FCM=15.58 +6.18Na +4.56K +2.73Cl -2.03Na^2 -1.99K^2 -6.90Cl^2 -3.71Na*K +4.22Na*Cl +4.11K*Cl	.80	21.51	Maximum	OHER[2]	OLER3[3]	.93
FAT=1.69 +2.73Na +1.07K +1.03Cl -1.40Na^2 -.24K^2 -.71Cl^2 -.72Na*K -.03Na*Cl -.03K*Cl	.87	3.45	Maximum	.67	1.18	.69
MP=3.48 -.42Na -.30K -.81Cl +.53Na^2 -.06K^2 -.03Cl^2 -.07Na*K -.10Na*Cl +.65K*Cl	.60	2.83	Saddle point	.57	1.43	.78
BWG=7.42 +3.01Na -6.07K -8.88Cl 3.84Na^2 +1.21K^2 +3.84Na^2 -.00Na*K +2.34Na*Cl +3.44K*Cl	.63	.90	Saddle point	.85	OLER[3]	OHER[2]

[1] Factor critical value is point on the response surface where slopes simultaneously equal 0, otherwise known as the stationary point. In a maximum or minimum type of surface these points would be at the maximum or minimum point on the surface, respectively.

[2] Outside high end of experimental range.

[3] Outside low end of experimental range.

TABLE A-2. Parameter estimates, R^2, response mean, surface type and factor critical value[1], of full quadratic models for blood hydrogen ion concentration (H^+,neq/l), bicarbonate (HCO_3^-, meq/L), partial pressure of CO_2, mm Hg), base-excess (BE, meq/L) and anion gap (ANGAP, meq/L) for chapter 3.

Parameter Estimates	R^2	Response Mean	Surface Type	Factor Critical Values (%) Na	K	Cl
H^+=71.8 -35.9Na -17.5K -4.3Cl +25.8Na^2 +5.4K^2 -2.8Cl^2 -.5Na*K +8.0Na*Cl +3.5K*Cl	.51	48.81	Saddle point	.57	1.36	.89
HCO_3^-=26.7 +11.0Na +2.6K -10.5Cl -8.3Na^2 -1.1K^2 +2.9Cl^2 -2.1Na*K +2.6Na*Cl +2.6K*Cl	.72	27.33	Saddle point	.59	1.58	.86
pCO_2=64.1 -11.6Na -6.4K -7.6Cl -8.3Na^2 +1.5K^2 -5.4Cl^2 +1.4Na*K +21.0Na*Cl +3.1K*Cl	.42	53.00	Saddle point	OHER[2]	OLER[3]	OHER[2]
BE=-3.70 +15.97Na +4.85K -7.96Cl -11.68Na^2 -1.75K^2 +2.65Cl^2 -2.06Na*K +.99Na*Cl +1.77K*Cl	.65	.71	Maximum	.59	1.49	.90
ANGAP=51.7 -52.2Na -31.6K -41.2Cl +49.5Na^2 +1.8K^2 +17.9Cl^2 +20.0Na*K -30.6Na*Cl +22.4K*Cl	.73	5.72	Saddle point	.50	1.31	.76

[1] Factor critical value is point on the response surface where slopes simultaneously equal 0, otherwise known as the stationary point. In a maximum or minimum type of surface these points would be at the maximum or minimum point on the surface, respectively.

[2] Outside high end of experimental range.

[3] Outside low end of experimental range.

TABLE A-3. Parameter estimates, R^2, response mean, surface type and factor critical value[1], of full quadratic models for plasma Na, (PNa), K (PK), Cl (PCl), Ca (PCa), and Mg (PMg) concentration (all in meq/L) for chapter 3.

Parameter Estimates	R^2	Response Mean	Surface Type	Factor Critical Values (%) Na	K	Cl
PNa=151.6 -13.7Na -28.9K -22.5Cl +27.4Na^2 +2.7K^2 +9.6Cl^2 +12.3Na*K -37.2Na*Cl +20.6K*Cl	.74	123.22	Saddle point	.49	1.27	.78
PK=6.61 -3.08Na -1.24K +.12Cl +4.29Na^2 +.56K^2 +.12Cl^2 -.79Na*K -1.14Na*Cl +.27K*Cl	.69	5.15	Minimum	.57	1.33	.71
PCl=77.8 +25.3Na -0.0K +32.3Cl -12.5Na^2 +2.3K^2 -12.4Cl^2 -5.5Na*K -9.2Na*Cl -5.1K*Cl	.78	95.25	Saddle point	.38	1.42	.87
PCa=6.01 -4.23Na -.95K 1.33Cl +3.60Na^2 +.18K^2 -.40Cl^2 +.86Na*K -1.21Na*Cl +.07K*Cl	.44	4.92	Saddle point	.61	1.02	.85
PMg=3.06 -1.98Na +.46K -.58Cl +2.03Na^2 -.11K^2 +.17Cl^2 -.09Na*K -.10Na*Cl +.31K*Cl	.36	2.12	Saddle point	.53	1.05	.90

[1] Factor critical value is point on the response surface where slopes simultaneously equal 0, otherwise known as the stationary point. In a maximum or minimum type of surface these points would be at the maximum or minimum point on the surface, respectively.

TABLE A-4. Parameter estimates, R^2, response mean, surface type and factor critical value[1], of full quadratic models for whole blood Na (WBNa), K (WBK), Cl (WBCl), Ca (WBCa), and Mg (WBMg) concentration (all in meq/L) for chapter 3.

Parameter Estimates	R^2	Response Mean	Surface Type	Factor Critical Values (%) Na	K	Cl
WBNa=96.0 - 6.5 Na - 4.9K +1.6Cl -13.3Na^2 -2.8K^2 -11.5Cl^2 +8.8Na*K +11.7Na*Cl +6.3K*Cl	.80	87.60	Saddle point	OHER[c]	OHER[c]	OHER[c]
WBK=16.8 -12.75Na -4.7K -.79Cl +9.1Na^2 +1.5K^2 -1.6Cl^2 +.08Na*K +2.7Na*Cl +1.3K*Cl	.73	10.23	Saddle point	.57	1.24	.77
WBCl=115.1 -42.8Na -31.3K -4.4Cl +27.9Na^2 +12.6K^2 +9.9Cl^2 +5.5Na*K +4.9Na*Cl -8.2K*Cl	.79	83.50	Minimum	.58	1.32	.63
WBCa=1.26 +.55Na +1.07K -.15Cl -.08Na^2 +.20K^2 +.44Cl^2 -.31Na*K -.14Na*Cl -.33K*Cl	.69	2.15	Saddle point	OHER[2]	OLER[3]	.66
WBMg=1.88 -.28Na +.01K -.48Cl +.57Na^2 -.03K^2 +.10Cl^2 -.28Na*K -.05Na*Cl +.25K*Cl	.81	1.61	Saddle point	.61	1.31	.89

[1] Factor critical value is point on the response surface where slopes simultaneously equal 0, otherwise known as the stationary point. In a maximum or minimum type of surface these points would be at the maximum or minimum point on the surface, respectively.

[2] Outside high end of experimental range.

[3] Outside low end of experimental range.

TABLE A-5. Parameter estimates, R^2, response mean, surface type and factor critical value[1], of full quadratic models for milk Na (MLNa), K (MLK), Cl (MLCl), Ca (MLCa), and Mg (MLMg) concentration (all in meq/L) for chapter 3.

Parameter Estimates	R^2	Response Mean	Surface Type	Factor Critical Values (%) Na	K	Cl
MLNa=23.2 +10.3Na +1.7K -10.1Cl $-6.4Na^2$ $+.34K^2$ $+6.6Cl^2$ -3.5Na*K +.39Na*Cl -.59K*Cl	.48	23.17	Saddle point	.54	1.02	.79
MLK=26.0 -6.6Na +10.7K +5.0Cl $+4.9Na^2$ $-4.9K^2$ $-4.4Cl^2$ +1.4Na*K -1.5Na*Cl +2.8K*Cl	.64	33.44	Saddle point	.61	1.46	.94
MLCl=30.6 -10.2Na +8.1K -8.7Cl $-1.0Na^2$ $-2.5K^2$ $+12.2Cl^2$ +5.9Na*K +.7Na*Cl -6.2K*Cl	.65	30.53	Saddle point	OHER[2]	OHER[2]	.84
MLCa=76.2 +10.8Na -24.8K -28.0Cl $-26.9Na^2$ $+2.8K^2$ $+3.8Cl^2$ +12.4Na*K +4.8Na*Cl +12.0K*Cl	.88	49.59	Saddle point	.65	1.70	.61
MLMg=7.96 -2.49Na +2.99K -.38Cl $+2.67Na^2$ $+.46K^2$ $+1.59Cl^2$ -.93Na*K -.32Na*Cl -.99K*Cl	.65	9.58	Saddle point	.80	1.66	.72

[1] Factor critical value is point on the response surface where slopes simultaneously equal 0, otherwise known as the stationary point. In a maximum or minimum type of surface these points would be at the maximum or minimum point on the surface, respectively.

[2] Outside high end of experimental range.

TABLE A-6. Least squares analysis of variance for dry matter intake (DMI, kg/d), milk yield (MY, kg/d), 3.5% fat-corrected milk (3.5% FCM, kg/d) yield, milk fat percentage (MF, %), milk protein (MP, %), and body weight gain (BWG, kg/d) for chapter 3.

Source	DMI		MY		3.5% FCM		MF		MP		BWG	
	df	MS	df	MS	df	MS	df	MS	df	MS	df	MS
Period	3	43.87*	3	773.96**	3	552.72**	3	1.81**	3	1.96**	3	0.71
Cow	47	243.98*	47	57.88**	47	62.17**	47	0.64**	47	0.17**	47	1.09
Na	1	1.80	1	9.69+	1	21.45*	1	0.33**	1	0.00	1	1.79
K	1	11.03**	1	6.43	1	8.25	1	0.12	1	0.24**	1	7.10**
Cl	1	8.48*	1	0.66	1	1.20	1	0.14+	1	0.27**	1	7.56**
Na × Na	...	...	...	...	...	...	1	0.14+	...	...	...	...
K × K	...	...	...	...	...	...	1	0.06	...	...	...	...
Cl × Cl	...	...	1	10.57+	1	14.53*	1	0.17+	...	...	...	...
Na × K	1	8.65*	...	...	...	...	1	0.17+	...	...	...	...
Na × Cl	1	10.15*	...	...	...	...	...	...	...	...	...	...
K × Cl	...	...	1	10.26+	1	11.30+	...	...	1	0.28**	1	7.95**
Error	136	1.54	136	3.41	135	4.24	134	0.05	137	0.02	137	0.93

** $P \leq .01$; * $P \leq .05$; + $P \leq .1$.

TABLE A-7. Least squares analysis of variance for blood hydrogen ion concentration ($[H^+]$, neq/L), bicarbonate (HCO_3^-, meq/L), base excess (BE, meq/L), anion gap (ANGAP, meq/L) and pCO_2 (mm Hg) for chapter 3.

Source	$[H^+]$		HCO_3		BE		ANGAP		pCO_2	
	df	MS	df	MS	df	MS	df	MS	df	MS
Period	3	34.51	3	35.91**	3	34.34**	3	418.19**	3	25.13
Cow	47	34.26**	47	4.12**	47	4.61**	47	64.12	47	65.60**
Na	1	9.56	1	3.17	1	9.78+	1	322.19+	1	118.68+
K	1	0.35	1	1.94	1	0.92	1	150.64	1	17.52
Cl	1	3.67	1	7.31+	1	5.61	1	19.33	1	97.62+
Na × Na	...	...	...	...	1	8.54+	...	...	...	...
K × K	...	...	...	...	...	...	...	...	...	...
Cl × Cl	...	...	1	5.34+	...	...	...	...	...	...
Na × K	...	...	...	...	...	...	...	...	...	...
Na × Cl	...	...	...	...	...	...	...	254.95+	1	106.87+
K × Cl	...	...	...	...	...	...	1	237.75+	...	...
Error	113	19.72	112	2.08	112	2.59	113	89.94	113	35.56

** $P \leq .01$; * $P \leq .05$; + $P \leq .1$.

TABLE A-8. Least squares analysis of variance for plasma Na (PNa, meq/L), plasma K (PK, meq/L), plasma Cl (PCl, meq/L), plasma Ca (PCa, meq/L), and plasma Mg (PMg, meq/L) for chapter 3.

Source	PNa		PK		PCl		PCa		PMg	
	df	MS	df	MS	df	MS	df	MS	df	MS
Period	3	226.53*	3	2.38**	3	490.48**	3	0.04	3	0.11
Cow	47	48.38	47	0.27*	47	16.81**	47	0.40	47	0.18**
Na	1	389.02*	1	1.24**	1	41.17*	1	0.95+	1	0.25
K	1	147.48	1	0.16	1	10.20	1	0.04	1	0.01
Cl	1	5.57	1	0.04	1	57.87*	1	0.25	1	0.05
Na × Na	...	...	1	1.04*	...	...	1	0.94+	1	0.26+
K × K	...	...	...	...	...	...	...	...	...	...
Cl × Cl	...	...	...	...	1	52.43*	...	...	...	...
Na × K	...	...	...	...	...	...	...	...	...	...
Na × Cl	1	352.23*	...	...	...	...	...	...	...	...
K × Cl	1	216.49+	...	...	...	...	...	...	...	...
Error	112	75.66	113	0.18	113	9.30	113	0.31	113	0.10

** $P \leq .01$; * $P \leq .05$; + $P \leq .1$.

TABLE A-9. Least squares analysis of variance for whole blood Na (WBNa, meq/L), whole blood K (WBK, meq/L), whole blood Cl (WBCl, meq/L), whole blood Ca (WBCa, meq/L), and whole blood Mg (WBMg, meq/L) for chapter 3.

Source	WBNa		WBK		WBCl		WBCa		WBMg	
	df	MS	df	MS	df	MS	df	MS	df	MS
Period	3	4770.47**	3	14.25**	3	60.56*	3	0.72	3	0.52**
Cow	47	63.41**	47	12.00**	47	32.30**	47	0.05	47	0.06**
Na	1	3.96	1	6.86*	1	1.22	1	0.02	1	0.00
K	1	70.84*	1	2.52	1	35.85	1	0.10	1	0.00
Cl	1	8.24	1	0.26	1	42.81+	1	0.01	1	0.03+
Na × Na	...	...	1	6.29*	...	...	...	...	1	0.02
K × K	...	...	1	3.13	1	74.95*	...	...	...	...
Cl × Cl	...	...	...	...	...	...	1	0.09	...	...
Na × K	...	...	...	...	...	...	...	...	1	0.02
Na × Cl	...	...	...	...	...	...	...	...	...	...
K × Cl	...	...	...	...	1	32.12	1	0.06	1	0.03+
Error	111	14.88	109	1.32	106	16.88	109	0.03	108	0.01

** $P \leq .01$; * $P \leq .05$; + $P \leq .1$.

TABLE A-10. Least squares analysis of variance for milk Na (MLNa, meq/L), milk K (MLK, meq/L), milk Cl (MLCl, meq/L), milk Ca (MLCa, meq/L), and milk Mg (MLMg, meq/L) for chapter 3.

Source	MLNa		MLK		MLCl		MLCa		MLMg	
	df	MS	df	MS	df	MS	df	MS	df	MS
Period	3	198.13**	3	29.60**	3	47.93*	3	1269.91**	3	98.64**
Cow	47	162.69**	47	43.93**	47	164.01**	47	53.54+	47	3.92**
Na	1	9.08	1	0.55	1	26.65	1	22.76	1	5.75**
K	1	0.52	1	25.56*	1	3.93	1	152.49*	1	2.06+
Cl	1	25.40	1	22.06*	1	70.73*	1	128.87+	1	2.25+
Na × Na	...	...	...	...	...	...	1	98.17+	...	...
K × K	...	...	1	25.07*	...	...	...	...	...	...
Cl × Cl	1	23.24	...	...	1	83.84*	...	...	...	...
Na × K	...	...	...	...	...	...	1	51.47	...	...
Na × Cl	...	...	...	...	...	...	...	...	...	...
K × Cl	...	...	...	...	...	...	1	97.07+	...	...
Error	137	12.89	137	4.46	137	14.81	135	38.40	138	0.78

** $P \leq .01$; * $P \leq .05$; + $P \leq .1$.

APPENDIX B
STATISTICAL TABLES FOR CHAPTER 4

TABLE B-1. Least squares analysis of variance for dry matter intake (DMI, kg/d), milk yield (MY, kg/d), 3.5% fat-corrected milk (3.5% FCM, kg/d) yield, milk fat percentage (MF, %), milk protein (MP, %), and body weight gain (BWG, kg/d) for chapter 4.

Source	DMI		MY		3.5% FCM		MF		MP		BWG	
	df	MS	df	MS	df	MS	df	MS	df	MS	df	MS
Cow	35	11.98**	35	70.37**	35	54.92	35	0.40	35	0.19**	35	0.19
Period	2	1.02	2	88.93**	2	36.21	2	0.64	2	0.52**	2	0.01
Treatment												
1 - 7 vs. 8	1	1.20	1	1.69	1	0.30	1	0.03	1	0.02	1	0.36
1 - 6 vs. 7	1	0.19	1	4.65	1	7.33	1	0.01	1	0.07*	1	0.05
4,5 vs. 7	1	0.36	1	3.28	1	5.87	1	0.01	1	0.06+	1	0.00
2,3,6 vs. 1,4,5	1	0.45	1	0.21	1	0.15	1	0.01	1	0.00	1	0.16
2,3 vs. 6	1	0.08	1	0.12	1	0.03	1	0.00	1	0.00	1	0.02
2 vs. 3	1	1.68	1	0.51	1	1.72	1	0.02	1	0.05+	1	1.45
4 vs. 5	1	0.52	1	0.87	1	0.62	1	0.00	1	0.00	1	0.53*
Error	62	0.97	62	2.93	62	4.86	62	0.12	62	0.01	62	0.23

** $P \leq .01$; * $P \leq .05$; + $P \leq .1$.

TABLE B-2. Least squares analysis of variance for blood hydrogen ion concentration ($[H^+]$, neq/L), bicarbonate (HCO_3^-, meq/L), base excess (BE, meq/L), anion gap (ANGAP, meq/L) and pCO_2 (mm Hg) for chapter 3.

Source	$[H^+]$		HCO_3		BE		ANGAP		pCO_2	
	df	MS	df	MS	df	MS	df	MS	df	MS
Cow	35	42.65**	35	6.18**	35	7.45**	35	11.94*	35	45.18**
Period	2	165.37**	2	52.56**	2	37.38**	2	120.31**	2	460.46**
Treatment										
1 - 7 vs. 8	1	12.37	1	3.53	1	6.01	1	13.17	1	0.72
1 - 6 vs. 7	1	0.76	1	12.77*	1	10.38+	1	9.39	1	39.84
4,5 vs. 7	1	1.44	1	9.67+	1	8.04	1	8.07	1	27.58
2,3,6 vs. 1,4,5	1	0.42	1	0.03	1	0.03	1	0.89	1	0.39
2,3 vs. 6	1	4.24	1	0.76	1	0.18	1	27.42+	1	14.20
2 vs. 3	1	31.59	1	0.56	1	3.12	1	0.22	1	16.66
4 vs. 5	1	0.53	1	0.99	1	0.97	1	4.05	1	3.92
Error	61	21.96	61	2.74	61	3.67	61	7.17	61	18.97

** $P \leq .01$; * $P \leq .05$; + $P \leq .1$.

TABLE B-3. Least squares analysis of variance for plasma Na (PNa, meq/L), plasma K (PK, meq/L), plasma Cl (PCl, meq/L), plasma Ca (PCa, meq/L), and plasma Mg (PMg, meq/L) for chapter 4.

Source	PNa		PK		PCl		PCa		PMg	
	df	MS	df	MS	df	MS	df	MS	df	MS
Cow	35	11.64	35	0.17*	35	7.25	35	0.07**	35	0.03**
Period	2	1.77	2	0.04	2	39.46**	2	0.08+	2	0.03
Treatment										
1 - 7 vs. 8	1	23.26*	1	0.10	1	0.47	1	0.03	1	0.00
1 - 6 vs. 7	1	2.09	1	0.20	1	1.38	1	0.01	1	0.00
4,5 vs. 7	1	1.21	1	0.30+	1	0.38	1	0.01	1	0.01
2,3,6 vs. 1,4,5	1	1.68	1	0.17	1	0.44	1	0.02	1	0.01
2,3 vs. 6	1	0.48	1	0.00	1	35.35*	1	0.00	1	0.06+
2 vs. 3	1	2.93	1	0.22	1	6.03	1	0.09+	1	0.05+
4 vs. 5	1	4.24	1	0.47*	1	0.07	1	0.00	1	0.00
Error	62	6.45	62	0.10	62	6.00	62	0.03	62	0.02

** $P \leq .01$; * $P \leq .05$; + $P \leq .1$.

TABLE B-4. Least squares analysis of variance for whole blood Na (WBNa, meq/L), whole blood K (WBK, meq/L), whole blood Cl (WBCl, meq/L), whole blood Ca (WBCa, meq/L), and whole blood Mg (WBMg, meq/L) for chapter 4.

Source	WBNa		WBK		WBCl		WBCa		WBMg	
	df	MS	df	MS	df	MS	df	MS	df	MS
Cow	35	26.42+	35	10.57**	35	25.33**	35	0.06**	35	0.03*
Period	2	226.39**	2	6.74**	2	252.73**	2	0.04	2	0.01
Treatment										
1 - 7 vs. 8	1	0.05	1	0.18	1	1.32	1	0.07	1	0.00
1 - 6 vs. 7	1	5.68	1	0.12	1	0.01	1	0.00	1	0.00
4,5 vs. 7	1	6.89	1	0.03	1	5.26	1	0.00	1	0.01
2,3,6 vs. 1,4,5	1	0.02	1	0.06	1	0.26	1	0.02	1	0.00
2,3 vs. 6	1	4.41	1	0.94	1	49.45*	1	0.03	1	0.01
2 vs. 3	1	49.42+	1	2.43*	1	53.42*	1	0.01	1	0.03
4 vs. 5	1	28.35	1	0.14	1	11.88	1	0.01	1	0.01
Error	62	17.56	62	0.51	62	11.51	62	0.03	62	0.02

** $P \leq .01$; * $P \leq .05$; + $P \leq .1$.

TABLE B-5. Least squares analysis of variance for milk Na (MLNa, meq/L), milk K (MLK, meq/L), milk Cl (MLCl, meq/L), milk Ca (MLCa, meq/L), and milk Mg (MLMg, meq/L) for chapter 4.

Source	MLNa		MLK		MLCl		MLCa		MLMg	
	df	MS	df	MS	df	MS	df	MS	df	MS
Cow	35	56.15**	35	37.06*	35	47.32**	35	73.97**	35	2.70**
Period	2	16.84**	2	18.05*	2	13.96	2	859.66**	2	7.85**
Treatment										
1 - 7 vs. 8	1	1.32	1	24.44*	1	9.13	1	15.01	1	0.79+
1 - 6 vs. 7	1	11.83+	1	1.40	1	0.02	1	16.56	1	0.13
4,5 vs. 7	1	24.06*	1	0.39	1	4.05	1	26.28	1	0.09
2,3,6 vs. 1,4,5	1	5.66	1	5.53	1	1.35	1	0.60	1	0.26
2,3 vs. 6	1	0.02	1	8.83	1	6.71	1	20.37	1	0.18
2 vs. 3	1	11.50+	1	0.50	1	0.01	1	1.04	1	0.00
4 vs. 5	1	12.66+	1	3.23	1	19.90	1	33.53+	1	0.06
Error	62	4.19	62	3.63	62	7.79	62	11.37	62	0.21

** $P \leq .01$; * $P \leq .05$; + $P \leq .1$.

APPENDIX C
STATISTICAL TABLES FOR CHAPTER 6

TABLE C-1. Least squares analysis of variance for blood variables; Phase I K, Cl and K x Cl models for chapter 6.[1]

Source	df	Mean Squares				
		Blood H^+	Blood iCa^2	Plasma K	Plasma Cl	Red blood cell K
		(neq/L)	(meq/L)	(meq/L)		
K	1	11.3269*	...	.2150+	...	126.264+
Cl	1	10.5914+	.0475*	...	17.799*	...
hour	1	...	.1891**	.3271*	...	...
hour x Cl	1	...	.0936**	...	...	...
hour x hour	1	...	.1860**	.4525**	...	...
hour x hour x hour	1	...	.1616**	.4041*	...	...
Residual[3]	219	2.7777	.0157	.0749	4.132	38.946

[1]Terms not shown were nonsignificant (P > .1). Models tested linear K, Cl, K x Cl, linear through quintic hour terms and all interactions.

[2]Ionized Ca.

[3]Used to test all terms.

+P < .1.

*P < .05.

**P < .01.

TABLE C-2. Least squares analysis of variance for blood variables; Phase I cation-anion difference [CAD; meq (Na + K - Cl) / 100 g diet DM] models for chapter 6.[1]

Source	df	Means Squares		
		Blood H^+	Blood iCa^2	Plasma Cl
		(neq/L)	--------(meq/L)------	
CAD	1	21.765**	.0046	18.272**
hour	1	...	.0922*	...
CAD x hour	1	...	.048+	...
hour x hour	1	...	.186+	...
hour x hour x hour	1	...	.1616**	...
Residual[3]	219	2.778	.0157	4.132

[1]Terms not shown were nonsignificant (P > .1). Full models tested linear through quadratic CAD terms, linear through quintic hour terms and all interactions.

[2]Ionized Ca.

[3]Used to test all terms.

+P < .1.

*P < .05.

**P < .01.

TABLE C-3. Least squares analysis of variance for urine variables; Phase I K, Cl and K x Cl models for chapter 6.[1]

Source	df	Mean squares				
		Urine H^+	Urine Na/ Creatinine	Urine K/ Creatinine	Urine Cl/ Creatinine	Urine Ca/ Creatinine
		(neq/L)	(mmol/mmol)			
K	1	...	.90	48.01	11.01	.195+
Cl	1	550.819*	...	...	.34	...
hour	1	...	4524.96**	3504.84**	104.5	...
hour x K	1	...	2410.43*	3031.48**	432.78	...
hour x Cl	1	...	...	...	1526.77**	...
hour x K x Cl	1	...	...	...	960.26*	...
hour x hour	1	...	2723.11*	...	450.26	...
hour x hour x hour	1	...	...	...	629.90+	...
Residual[2]	144[3]	112.023	626.46	370.98	221.46	.059

[1]Terms not shown were nonsignificant ($P > .1$). Full models tested linear K, Cl, K x Cl, linear through quintic hour terms and all interactions.

[2]Used to test all terms.

[3]Residual df equaled 144 for Na/creatinine and Ca/creatinine; 143 for K/creatinine and Cl/creatinine; 139 for H^+.

+$P < .1$.

*$P < .05$.

**$P < .01$.

TABLE C-4. Least squares analysis of variance for urine variables; Phase I cation-anion difference [CAD; meq (Na + K -Cl) / 100 g diet DM] models for chapter 6.[1]

		Mean Squares		
Source	df	Urine H^+	Urine K/ Creatinine	Urine Ca/ Creatinine
		(neq/L)		
CAD	1	533.532*	.613	.282*
hour	1	...	474.735	...
CAD x hour	1	...	1557.870*	...
Residual[2]	139[3]	112.023	370.979	.059

[1]Terms not included were nonsignificant (P > . 1). Models tested linear through quadratic CAD terms, linear through quintic hour terms and all interactions.

[2]Used to test all terms.

[3]Residual df equaled 139 for H^+; 143 for K/creatinine and Ca/creatinine.

+P < .1.

*P < .05.

**P < .01.

TABLE C-5. Least squares analysis of variance for blood variables; Phase II K, Cl and K x Cl models for chapter 6.[1]

Source	df	Mean Squares			
		H^+	iCa^2	Plasma Cl	Plasma Protein
		(neq/L)	(meq/L)	(meq/L)	(mg/dl)
Cl	1	9.758*	.0063**	25.154**	.3776+
day	1	...	...	7.853+	...
Residual[3]	140[4]	1.559	.0009	2.529	.0999

[1]Terms not shown were nonsignificant (P >.1). Models tested linear K, Cl, K x Cl, linear through cubic day terms and all interactions.

[2]Ionized Ca.

[3]Used to test all terms.

[4]Residual df equaled 140 for H^+, iCa, and plasma Cl; and 138 for plasma protein.

TABLE C-6. Least squares anlaysis of variance for blood variables; Phase II cation-anion difference [CAD; meq (Na + K - Cl) / 100 g diet DM] models for chapter 6.[1]

		Mean Squares				
Source	df	Blood H^+	Plasma HCO_3^-	Blood iCa[2]	Plasma Cl	Plasma Protein
		(neq/L)	----------(meq/L)----------			(mg/dl)
CAD	1	10.094*	7.751+	.0057**	18.27*	.312+
Residual[3]	140[4]	1.559	2.365	.0009	2.53	.0999

[1]Terms not shown were nonsignificant (P > .1). Models tested linear through quadratic CAD terms, linear through cubic day terms and all interactions.

[2]Ionized Ca.

[3]Used to test all terms.

[4]Residual df equaled 140 for blood H^+, HCO_3^-, iCa, and plasma Cl; 138 for plasma protein.

+P < .1.

*P < .05.

**P < .01.

TABLE C-7. Least squares analysis of variance for urine variables; Phase II K, Cl and K x Cl models for chapter 6.[1]

Source	df	Mean Squares: Urine Na/ Creatinine	Urine K/ Creatinine	Urine Cl/ Creatinine	Urine Ca/ Creatinine
		(mmol/mmol)			
K	1	224.41	10521.01**	488.98	.2092+
Cl	1	106.33	...	3299.49**	...
K x Cl	1	2462.97*	...	1011.72*	...
day	1	355.89	...	...	...
day x K	1	80.77	...	...	...
day x Cl	1	248.14	...	...	...
day x K x Cl	1	2628.61*	...	...	...
Residual[2]	139[3]	591.95	338.26	214.56	.0762

[1]Terms not shown were nonsignificant (P > .1). Models tested linear K, Cl, K x Cl, linear through cubic day terms and all time by mineral interactions.

[2]Used to test all terms.

[3]Residual df equaled 139 for Na/creatinine, K/creatinine, Cl/creatinine and Ca/creatinine.

+P < .1.

*P < .05.

**P < .01.

TABLE C-8. Least squares anlaysis of variance for urine variables; Phase II cation-anion difference [CAD; meq (Na + K - Cl) / 100 g diet DM] models for chapter 6.[1]

		Mean Squares				
Source	df	Urine H+	Urine Na/ Creatinine	Urine K/ Creatinine	Urine Cl/ Creatinine	Urine Ca/ Creatinine
		(meq/L)	(mmol/mmol)			
CAD	1	2774.04**	2476.48*	8274.15**	843.36*	0.2978*
CAD x CAD	1	...	2421.16*	...	970.51*	...
day	1	...	1884.99+	...	...	...
CAD x day	1	...	2400.07*	...	...	...
CAD x CAD x day	1	...	2649.41*	...	...	...
Residual[2]	139[3]	559.58	591.95	338.26	214.56	.0762

[1]Terms not shown were nonsignificant (P > .1). Models tested linear through quadratic CAD terms, linear through cubic day terms and all interactions.

[2]Used to test all terms.

[3]Residual df equaled 139 for Na/creatinine, K/creatinine, Cl/creatinine and Ca/creatinine.

+P < .1.

*P < .05.

**P < .01.

TABLE C-9. Least squares analysis of variance for mineral digestion coefficients; Phase II models for chapter 6.[1]

		Mean Squares							
		K, Cl, K x Cl Models[2]				CAD Models[3]			
Source	df	K[4]	K[5]	Cl[4]	Cl[5]	K[4]	K[5]	Cl[4]	Cl[5]
		(% Digestibility)							
K	1	127.67**	108.89**	...	...				
Cl	1	...	...	1883.87**	1505.82**				
Dry matter intake	1		.62		26.68				
CAD	1					64.20*	47.45*	443.52*	96.74
Dry matter intake	1						2.65		57.94
Residual	140	14.83	14.83	83.48	83.48	14.83	14.83	83.48	83.48

[1]Terms not shown were nonsignificant (P > .1).

[2]Models tested linear K, Cl, K x Cl, linear through cubic day terms and all interactions.

[3]CAD = Cation-anion difference [meq (Na + K - Cl) / 100 g diet DM. Models tested linear through quadratic CAD terms, linear through cubic day terms and all interactions.

[4]Fit without dry matter intake as covariate.

[5]Fit with dry matter intake as covariate.

[6]Used to test all terms.

*P < .05.

**P < .01.

TABLE C-10. Least squares analysis of variance for milk composition; Phase II models for chapter 6.[1]

Source	df	Mean Squares	
		Milk H+	Milk Cl
		(neq/L)	(meq/L)
K	1	2502.17**	...
Cl	1	4088.60**	41.43+
Residual[2]	140[3]	368.90	13.70

[1]Terms not shown were nonsignificant (P > .1). One set of models tested linear K, Cl, K x Cl, linear through cubic day terms and all interactions. A second set of models tested linear through quadratic cation-anion difference [meq (Na + K - Cl) / 100 g diet DM] terms, linear through cubic day terms and all interactions.

[2]Used to test all terms.

[3]Residual df equaled 140 for milk Cl and 158 for milk H^+ concentration.

+P < .1.

**P < .01.

TABLE C-11. Least squares analysis of variance for body temperature; Phase II K, Cl and K x Cl models for chapter 6.[1]

Source	df	Mean Squares: Body temperature without DMI[2]	Mean Squares: Body temperature with DMI[3]
		--------(° C)-------	
Cl	1	.2820+	.3125*
day	1	.3365*	.3722*
day x day	1	.4222*	.4614*
DMI	1		.0463
Residual[4]	134	.0762	.0762

[1]Terms not shown were nonsignificant (P > .1). Models tested linear K, Cl, K x Cl, linear through cubic day terms and all interactions.

[2]Fit without DMI as covariate.

[3]Fit with DMI as covariate.

[4]Used to test all terms.

+P < .1.

*P < .05.

REFERENCES

Adams, 1975. Variability in mineral and trace element content of dairy cattle feeds. J. Dairy Sci. 58:1538.

Agricultural Research Council. 1980. Nutrient requirements of ruminant livestock. Commonwealth Agricultural Bureaux., Lavenham Press Ltd., Lavenham, Suffolk, U.K.

Aitken, F.C. 1976. Sodium and potassium nutrition of mammals. Tech. Comm. No. 26. Commonwealth Agricultural Bureaux of Nutrition. Bucksburn, Aberdeen, U.K.

Austic, R.E. 1988. Dietary mineral balance: Its relationship to acid-base homeostasis in poultry. Page 2 in Proc of Calif. Anim. Nutr.

Beede, D.K. 1991. Mineral and water nutrition. Vet. Clinics:North America. 7:373.

Beede, D.K., J.V. Briceno, and C.R. Staples. 1987. Lactational performance of mid-lactation Holstein cows fed two diet types with varying contents of refined trona or sodium bicarbonate. J. Dairy Sci. 70 (Suppl. 1):199

Beede, D.K., P.L. Schneider, P.G. Mallonee, R.J. Collier, and C.J. Wilcox. 1983. Potassium nutrition and the relationship with heat stress in lactating dairy cattle. Page 1 in Proc. 6th Ann. Int. Minerals Conf. Mundellein, IL.

Block, E. 1984. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. J. Dairy Sci. 67:2939.

Bolenbaugh, D.L. 1977. The effects of various dietary levels of potassium on subsequent parturition and lactation in the dairy cow. M.S. Thesis, Michigan State Univ., East Lansing.

Boron, W.F. 1986. Intracellular pH regulation. Page 423 in Physiology of membrane disorders. T.E. Andreoli, J.K. Hoffmann, D.D. Fanestil and S.G. Schultz. eds. Plenum Medical Book Company, New York.

Briceno, J.V., H.H. Van Horn, B. Harris Jr., and C.J. Wilcox. 1987. Effects of neutral detergent fiber and roughage source on dry matter intake and milk yield and composition of dairy cows. J. Dairy Sci. 70:298.

Briceno, J.V., H.H. Van Horn, B. Harris Jr., and C.J. Wilcox. 1988. Comparison of different methods of expressing dietary protein for lactating dairy cows. J. Dairy Sci. 71:1647.

Brown, C.A., P.T. Chandler, and J.B. Holter. 1977. Development of predictive equations for milk yield and dry matter intake in lactating cows. J. Dairy Sci. 60:1739.

Brown, C.A., C.C. Stallings, and S.W. Telega. 1980. Symposium: Systems analysis to establish managerial goals in dairy production. Nutritional modeling and its impact on managerial goals in dairy production. J. Dairy Sci. 64:2083.

Burns, C.H., W.W. Cravens, and P.H. Phillips. 1953. The sodium and potassium requirements of the chick and their interrelationships. J. Nutr. 50:317.

Campbell, L.D. and W.K. Roberts. 1965. The requirements and role of potassium in ovine nutrition. Can. J. Anim. Sci. 45:147.

Chandler, P.T. 1988. Chloride: An often overlooked mineral for dairy cows. Feedstuffs. Page 13. Feb. 22, 1988.

Chaney, A.L. and E.P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130.

Cochran, W.G. and G. Cox. 1957. Pages 135 through 140 in Experimental designs (2nd Ed.). John Wiley and Sons, Inc., New York.

Collier, R.J., D.K., Beede, W.W. Thatcher, L.A. Israel, and C.J. Wilcox. 1982. Influence of environment and its modification on dairy animal health and production. J. Dairy Sci. 65:2213.

Coppock, C.E. 1986. Mineral utilization by the lactating cow--chlorine. J. Dairy Sci. 69:595.

Coppock, C.E., R.A. Aguirre, L.E. Chase, G.B. Lake, R.E. McDowell, M.J. Fettman, and M.E. Woods. 1979. Effects of a low chloride diet on lactating Holstein Cows. J. Dairy Sci. 62:723.

Coppock, C.E. and M.J. Fettman. 1977. Chloride as a required nutrient for lactating dairy cows. Page 43 in Proc. Cornell Nutr. Conf. Ithaca, NY.

Coppock, C.E., P.A. Grant, S.J. Portzer, and A. Escobosa. 1982a. Effect of varying dietary ratio of sodium and chloride on the response of lactating dairy cows in hot weather. J. Dairy Sci. 65:552.

Coppock, C.E., P.A. Grant, S.J. Portzer, and A. Escobosa. 1982b. Effect of differences in dietary sodium, chloride and bicarbonate on responses of lactating dairy cows in hot weather. J. Dairy Sci. 65:566.

Cornell, J.A. 1990. Experiments with mixtures: Designs, models, and the analysis of mixture data. 2nd ed. John Wiley, New York.

Cotlove, E. 1963. Determination of the true chloride content of biological fluids and tissues. II: Analysis by simple, nonisotopic methods. Anal. Chem. 35:95.

Croom, W.J., Jr., R.W. Harvey, A.C. Linnerud, and M. Froetschel. 1982. High levels of sodium chloride in beef cattle diets. Can. J. Anim. Sci. 62:217.

Dean, R.B. 1941. Theories of electrolyte equilibrium in muscle. Biol. Symp. 3:331.

de Groot, T.H. and J.H. Aafjes. 1960. On the constancy of creatinine excretion in the urine of the dairy cow. Br. Vet J. 116:409.

Dennis, R.J. and R.W. Hemken. 1978. Potassium requirements of dairy cows in early and mid-lactation. J. Dairy Sci. 61:757.

Dennis, R.J., R.W. Hemken, and D.R. Jacobson. 1976. Effects of dietary potassium percentage for lactating dairy cows. J. Dairy Sci. 59:324.

Dils, R.R. 1989. Synthetic and secretory process of lactation. Proc. Nutr. Soc. 48:9.

Dishington, I.W. 1975. Prevention of milk fever (hypocalcemia paresis puerperalis) by dietary salt supplementation. Acta. Vet. Scand. 16:503.

Downer, J.V. and K.R. Cummings. 1987. Sodium bicarbonate: a ten year review of lactation studies. J. Dairy Sci. 70:(Suppl 1):152.(Abstr.).

Dweyer, J.E. Foulkes, M. Evans, and L. Ausman. 1985. Acid/Alkaline ash diets: Time for assessment and change. J. Am. Dietetic Assn. 85:841.

Edelman, I.S., A.H. James, H. Boden, and F.D. Moore. 1954. Electrolyte composition of bone and the penetration of radiosodium and deuterium oxide into dog and human bone. J. Clin. Invest. 33:122.

Eicker, S.W. 1990. An introduction to strong ion difference. Vet. Clinics of North America: Food Animal Practice. 6:45.

Erdman, R.A. 1988. Dietary buffering requirements of the lactating dairy cow: A review. J. Dairy Sci. 71:3246.

Erdman, R.A., R.W. Hemken, and L.S. Bull. 1980. Effects of dietary calcium and sodium on potassium requirements for lactating dairy cows. J. Dairy Sci. 63:538.

Erdman, R.A., R.W. Hemken, and L.S. Bull. 1982. Dietary sodium bicarbonate and magnesium oxide for early postpartum lactating dairy cows: Effects on production, acid-base metabolism, and digestion. J. Dairy Sci. 65:712.

Escobosa, A., C.E. Coppock, L.D. Rowe J.R., and C.E. Gates. 1984. Effects of dietary sodium bicarbonate and calcium chloride on physiological responses of lactating dairy cows in hot weather. J. Dairy Sci. 67:574.

Fettman, M.J., L.E. Chase, J. Bentinck-Smith, C.E. Coppock, and S.A. Zinn. 1984a. Restricted dietary chloride with sodium bicarbonate supplementation for Holstein cows in early lactation. J. Dairy Sci. 67:1457.

Fettman, M.J., L.E. Chase, J. Bentinck-Smith, C.E. Coppock, and S.A. Zinn. 1984b. Nutritional chloride deficiency in early lactation Holstein cows. J. Dairy Sci. 67:2321.

Fischer, J.L., R.F. Husted, and P.R. Steinmetz. 1983. Chloride dependence of the HCO_3 exit step in urinary acidification by the turtle bladder. Am. J. Physiol. 245:F627.

Fontenot, J.P., R.W. Miller, C.K. Whitehair, and R. MacVicar. 1960. Effect of a high-protein high-potassium ration on the mineral metabolism of lambs. J. Anim. Sci. 19:127.

Fredeen, A.H., E.J. DePeters, and R.L. Baldwin. 1988. Effects of acid-base disturbances caused by differences in dietary fixed ion balance on kinetics of calcium metabolism in ruminants with high calcium demand. J. Anim. Sci. 66:174.

Golz, D.I. and T.D. Crenshaw. 1990. Interrelationships of dietary sodium, potassium and chloride on growth in young swine. J. Anim. Sci. 68:2736.

Grunert, R.R., J.H. Meyer, and P.H. Phillips. 1950. The sodium and potassium requirement of the rat for growth. J. Nutr. 42:609.

Harrison, D.G., D.E. Beever, D.J. Thomson, and D.F. Osborn. 1975. Manipulation of rumen fermentation in sheep by increasing the rate of flow of water from the rumen. J. Sci. Food Agric. 85:93.

Harrison, D.G., D.E. Beever, D.J. Thomson, and D.F. Osborn. 1976. Manipulation of fermentation in the rumen. J. Sci. Food Agric. 27:617.

Hemken, R.W. 1983. Potassium in ruminant nutrition. Page 1 in Sodium and potassium in animal nutrition. National Feed Ingredients Association, West Des Moines, IA.

Henry, R.J., D.C. Cannon, and J.W. Winkelman. 1974. Page 521 in Clinical chemistry: Principles and techniques. 2nd ed. Harper and Row Inc., Hagerston, MD.

Herod, E.L., R.M. Bechtle, E.E. Bartley, and A.D. Dayton. 1978. Buffering ability of several compounds in vitro and the effect of a selected buffer combination on ruminal acid production in vivo. J. Dairy Sci. 61:1114.

Hilwig, R.V. 1976. Excretion and renal regulation of neutrality. Page 19 in Buffers in ruminant physiology and metabolism. M.S. Weinberg and A.L. Sheffner, eds. Church and Dwight, Inc., New York.

Hurwitz, S., A. Cohen, and S. Bornstein. 1973. Sodium and chloride requirements of the chick; relationship to acid-base balance. Poult. Sci. 52:903.

Jackson, H.M., R.P. Kromann, and E.E. Ray. 1971. Energy retention in lambs as influenced by various levels of sodium and potassium in the rations. J. Anim. Sci. 33:872.

Jacobson, D.R., R.W. Hemken, F.S. Button and R.H. Hatton. 1972. Mineral nutrition, calcium, phosphorous, magnesium and potassium interrelationships. J. Dairy Sci. 55:935.

Johnson, R.J. and H. Karunajeewa. 1985. The effect of dietary minerals and electrolytes on the growth and physiology of the young chick. J. Nutr. 115:1680.

Kellaway, R.C., D.J. Thomson, D.E. Beever, and D.F. Osborn. 1977. Effects of NaCl and $NaHCO_3$ on food intake, growth rate and acid-base balance in calves. J. Agric. Sci. Camb. 88:1.

Khuri, A.I. and J.A. Cornell. 1987. Response surfaces: Designs and analyses. Marcel Dekker, Inc. New York, NY.

Kilmer, L.H., L.D. Muller, and P.J. Wangsness. 1981. Addition of sodium bicarbonate to rations of pre- and postpartum dairy cows. J. Dairy Sci. 63:2026.

Kleinman, L.I. and J.M. Lorenz. 1989. Physiology and pathophysiology of body water and electrolytes. Chapter 20 in Clinical chemistry: Theory analysis and correlation. L.A. Kaplan and A.J. Pesce, eds. The C.V. Mosby Company. St. Louis, Baltimore, Philadelphia, Toronto.

Kronfeld, D.S. 1976. Metabolic and respiratory adjustments of acid-base balance and the burden of exogenous acid in ruminants. Page 41 in Buffers in ruminant physiology and metabolism. M.S. Weinberg and A.L. Sheffner, eds. Church and Dwight, Inc., New York.

Kronfeld, D.S. 1979. Sodium, osmolarity and hydration. Page 12 in Regulation of acid-base balance. W.H. Hale and P. Meinhardt, eds. Church and Dwight, Inc. Piscataway, NJ.

Kumpost, H.E. and T.W. Sullivan. 1966. Minimum Na requirement and interaction of K and Na in the diet of young turkeys. Poult. Sci. 45:1334.

Leach, R.M. 1979. Dietary electrolytes: Story with many facets. Feedstuffs. April 30, p. 27.

Lechene, C. 1988. Physiological role of the Na-K pump. Page 171 in The Na, K-pump, part B: Cellular Aspects. Alan R. Liss, Inc.

Linsner, J.R. 1980. Potassium. Anim. Nutr. and Health. Oct., Page 1.

Lomba, F., G. Chauvaux, E. Teller, L. Lengels, and V. Bienfet. 1978. Calcium digestibility in cows as influenced by the excess of alkaline ions over stable acid ions in their diets. Br. J. Nutr. 39:425.

Lough, D.S., D.K. Beede, and C.J. Wilcox. 1990. Lactational responses to and in vitro ruminal solubility of magnesium oxide or magnesium chelate. J. Dairy Sci. 73:413.

Lunn, D.P. and S.M. McGuirk. 1990. Renal regulation of electrolyte and acid-base balance in ruminants. Vet. Clinics of North America: Food Animal Practice. 6:1.

Madshus, I.H. 1988. Regulation of intracellular pH in eukaryotic cells. Biochem. J. 250:1.

Mallonee, P.G., D.K. Beede, R.J. Collier, and C.J. Wilcox. 1982. Lactational and physiological responses of dairy cows to varying potassium and sodium quantities and ratios in complete mixed diets. J. Dairy Sci. 65 (Suppl. 1):212.

Mallonee, P.G., D.K. Beede, R.J. Collier, and C.J. Wilcox. 1985. Production and physiological responses of dairy cows to varying dietary potassium during heat stress. J. Dairy Sci. 68:1479.

Mann, C. 1990. Meta-analysis in the breech. Science. 249:476.

Martens, H. and I. Blume. 1987. Studies on the absorption of sodium and chloride from the rumen of sheep. Comp. Biochem. Physiol. 86A:653.

Mattill, H.A. and H.I. Mattill. 1922. Mineral metabolism. Page 303 in Endocrinology and metabolism. L.F. Barker ed. Appleton Co. New York.

McGuirk, S.M. and D.G. Butler. 1980. Metabolic alkalosis with paradoxic aciduria in cattle. J. Am. Vet. Med. Assn. 177:551.

McKeown, J.W. 1986. Disorders of Na metabolism. Page 63 in Fluids and electrolytes. J.P Kakko and R.L Tannen ed. W.P. Saunders Co. Philadelphia.

Miller, W.J. 1981. Mineral and vitamin nutrition of dairy cattle. J. Dairy Sci. 64:1196.

Mongin, P. 1980. Electrolytes in nutrition: review of basic principles and practical application in poultry and swine. Page 1 in Proc. 3rd Ann. Int. Minerals Conf., Mundellein, IA.

Montgomery. D.C. 1984. Design and analysis of experiments. 2nd ed. John Wiley and Sons, Inc. New York.

Moore, C.W. 1970. Ionized calcium in normal ultrafiltrate and whole blood determined by ion exchange electrodes. J. Clin. Invest. 49:318.

Morris, J.G. 1986. Overview of acid-base metabolism. Page 1 in Proc. 9th Ann. Int. Minerals Conf., Mundellein, IA.

Morse, D., H.H. Head, and B. Harris, Jr. 1989. Dietary phosphorous and calcium effects on lactational performance of Holstein cows. J. Dairy Sci. 72 (Suppl. 1):476.

Murphy, M.R. 1982. Analyzing and presenting pH data. J. Dairy Sci. 65:161.

National Research Council. 1971. Nutrient requirements of dairy cattle. 4th ed. Nat'l. Acad. Sci., Washington, D.C.

National Research Council. 1978. Nutrient requirements of dairy cattle. 5th ed. Nat'l. Acad. Sci., Washington, D.C.

National Research Council. 1980. Mineral tolerance of domestic animals. Nat'l. Acad. Sci., Washington, D.C.

National Research Council. 1989. Nutrient requirements of dairy cattle. 6th rev. ed. Nat'l. Acad. Sci., Washington, D.C.

Neilsen, R. 1979. A 3 to 2 coupling of the Na-K pump in frog skin. Acta Physiol. Scand. 107:189.

Nelson, A.B., R.W. MacVicar, Jr., and J.C. Meiske. 1955. Effect of a high salt intake on the digestibility of ration constituents and on nitrogen, sodium and chloride retention by steers and wethers. J. Anim. Sci. 14:825.

Nestor, K.E., R.W. Hemken, and R.J. Harmon. 1988. Influence of sodium chloride and potassium bicarbonate on udder edema and selected blood parameters. J. Dairy Sci. 71:366.

O'Connor, A.M., D.K. Beede, and C.J. Wilcox. 1988. Lactational responses to dietary magnesium, potassium, and sodium during winter in Florida. J. Dairy Sci. 71:971.

O'Dell, B.L. and J.E. Savage. 1966. Arginine-lysine antagonism in the chick and its relationship to dietary cations. J. Nutr. 90:364.

Odom, T. 1989. Thin egg shells in hot weather: A matter of survival. Feedstuffs. April 24, p. 20.

Oetzel, G.R. 1991. Meta-analysis of nutritional risk factors for milk fever in dairy cattle. J. Dairy Sci. 74:3900.

Oetzel, G.R., J.D. Olson, C.R. Curtis, and M.J. Fettman. 1988. Ammonium chloride and ammonium sulfate for prevention of parturient paresis in dairy cows. J. Dairy Sci. 71:3302.

Papas, A.M., S.R. Ames, R.M. Cook, C.J. Sniffen, C.E. Polan, and L. Chase. 1984. Production responses of dairy cows fed diets supplemented with ammonium salts of iso C-4 and C-5 acids. J. Dairy Sci. 67:276.

Paquay, R., F. Lomba, A. Lousse, and V. Bienfet. 1968. Statistical research on the fate of dietary mineral elements in dry and lactating cows. I. Calcium. J. Agric. Sci. 71:173.

Paquay, R., F. Lomba, A. Lousse, and V. Bienfet. 1969a. Statistical research on the fate of dietary mineral elements in dry and lactating cows. IV. Chloride. J. Agric. Sci. 73:223.

Paquay, R., F. Lomba, A. Lousse, and V. Bienfet. 1969b. Statistical research on the fate of dietary mineral elements in dry and lactating cows. V. Potassium. J. Agric. Sci. 73:445.

Patience, J.F., R.E. Austic, and R.D. Boyd. 1987. Effect of dietary electrolyte balance on growth and acid-base status of swine. J. Anim. Sci. 64:457.

Peeler, H.T. 1972. Biological availability of nutrients in feeds: availability of major mineral ions. J. Anim. Sci. 35:695.

Pradhan, K. and R.W. Hemken. 1968. Potassium depletion in lactating dairy cows. J. Dairy Sci. 51:1377.

Rogers, J.A., C.L. Davis, and J.H. Clark. 1979. Alteration of rumen fermentation in steers by increasing rumen fluid dilution rate with mineral salts. J. Dairy Sci. 62:1599.

Rogers, J.A., C.L. Davis, and J.H. Clark. 1982a. Alteration of rumen fermentation, milk fat synthesis, and nutrient utilization with mineral salts in dairy cows. J. Dairy Sci. 65:577.

Rogers, J.A., C.L. Davis, and J.H. Clark. 1982b. Effects of intraruminal infusion of mineral salts on volatile fatty acid production in steers fed high-grain and high-roughage diets. J. Dairy Sci. 65:953.

St. Omer, V.V.E., and W.K. Roberts. 1967. Some effects of dietary potassium upon digestibility, serum electrolytes and utilization of potassium, sodium, nitrogen and water in heifers. Can. J. Anim. Sci. 47:39.

Sanchez, W.K. and D.K. Beede. 1991. Interrelationships of dietary Na,K and Cl and cation-anion difference in lactation rations. Page 31 in Proc. Florida Rum. Nutr. Conf. University of Florida, Gainesville.

Sanchez, W.K., D.K. Beede, and J.A. Cornell. 1990a. Lactational performance and acid-base status of midlactation Holsteins fed graded concentrations of sodium, potassium and chloride. J. Dairy Sci. 73:(Suppl. 1):162 (Abstr.).

Sanchez, W.K., D.K. Beede, J.A. Cornell, S.L. Means, and T.M. Ruiz. 1990b. Effects of dietary mixtures of sodium bicarbonate, sodium chloride and potassium chloride on lactational performance and acid-base status of midlactation Holsteins. J. Dairy Sci. 73:(Suppl. 1):162.

Sanchez, W.K., D.K. Beede, and M.A. DeLorenzo. 1991. Modeling the effects of macrominerals and their interrelationships of lactational performance of dairy cattle: A compilation of data from ten experiments conducted in Florida during the 1980s. J. Dairy Sci. 74:(Suppl. 1):191 (Abstr.).

SAS. 1985. SAS Users guide: Statistics. SAS Institute, Inc. Cary, NC.

Schneider, B.H. and W.P. Flatt. 1975. Page 168 in The evaluation of feeds through digestibility experiments. Univ. Georgia Press, Athens.

Schneider, P.L., D.K. Beede, and C.J. Wilcox. 1984a. Nycterohemeral patterns of acid-base status, mineral concentrations and digestive function of lactating cows in natural or chamber heat environments. J. Anim. Sci. 66:112.

Schneider, P.L., D.K. Beede, and C.J. Wilcox. 1986. Responses of lactating cows to dietary sodium source and quantity and potassium quantity during heat stress. J. Dairy Sci. 69:99.

Schneider, P.L., D.K. Beede, C.J. Wilcox, and R.J. Collier. 1984b. Influence of dietary sodium and potassium bicarbonate and total potassium on heat-stressed lactating dairy cows. J. Dairy Sci. 67:2546.

Scott, D. 1967. The effects of potassium supplements upon the absorption of potassium of potassium and sodium from the rumen of sheep. Q. J. Exp. Physiol. 52:382.

Scott, D. 1970. Aspects of renal function in ruminants. Rowett Res. Inst. Ann. Rep. 26:98.

Shohl, A.T. 1939. Mineral metabolism. Reinhold Publishing Corp, New York.

Shohl, A.T. and A. Sato. 1923. Acid-base metabolism. I. Determination of base balance. J. Biol. Chem. 58:235.

Skou, J.C. 1957. The influence of some cations on an adenosine triphosphate from peripheral nerves. Biochem. Biophys. Acta 23:394.

Sperber, I. and S. Hyden. 1952. Transport of chloride through the ruminal mucosa. Nature. 169:587.

Stacy, B.D. and B.W. Wilson. 1970. Acidosis and hypercalciuria: renal mechanisms affecting calcium, magnesium, and sodium excretion int he sheep. J. Physiol. 210:549.

Staples, C.R., S.M. Emanuele, M. Ventura, D.K. Beede, and B.R. Schricker. 1988. Effects of a new multielement buffer on production, ruminal environment, and blood minerals of lactating dairy cows. J. Dairy Sci. 71:1573.

Staples, C.R. and D.S. Lough. 1989. Efficacy of supplemental dietary neutralizing agents for lactating dairy cows. A review. Anim. Feed Sci. Technol., 23:277-303.

Stewart, P.A. 1981. How to understand acid-base: A quantitative acid-base primer for biology and medicine. Elsevier, New York.

Suttle, N.F. and A.C. Field. 1967. Studies on magnesium in ruminant nutrition: Effect of increased intakes of potassium and water on the metabolism of magnesium, phosphorous, sodium, potassium and calcium in sheep. Brit. J. Nutr. 21:819.

Tucker, W.B., G.A. Harrison, and R.W. Hemken. 1988a. Influence of dietary cation-anion balance on milk, blood, urine, and rumen fluid in lactating dairy cattle. J. Dairy Sci. 71:346.

Tucker, W.B., and J.F. Hogue. 1990. Influence of sodium chloride or potassium chloride on systemic acid-base status, milk yield, and mineral metabolism in lactating dairy cows. J. Dairy Sci. 73:3485.

Tucker, W.B., Z. Xin, and R.W. Hemken. 1988b. Influence of dietary calcium chloride an adaptive changes in acid-base status and mineral metabolism in lactating dairy cows fed a diet high in sodium bicarbonate. J. Dairy Sci. 71:1587.

Underwood, E.J. 1981. The mineral nutrition of livestock. Commonwealth Agricultural Bureaux, London.

United States Department of Agriculture. 1990. Economic Research Service. Washington, D.C.

Wang, C. and D.K. Beede. 1992. Effects of ammonium chloride and sulfate on acid-base status and calcium metabolism of dry cows. J.Dairy Sci. (in press).

Ward, G.M. 1966. Potassium metabolism of domestic ruminants--A review. J. Dairy Sci. 49:268.

West, J.W., C.E. Coppock, D.H. Nave, J.M. Labore, L.W. Greene, and T.W. Odom. 1987a. Effects of potassium carbonate and sodium bicarbonate on rumen function in lactating holstein cows. J.Dairy Sci. 70:81.

West, J.W., C.E. Coppock, K.Z. Milam, D.H. Nave, J.M. Labore, and L.D. Rowe. 1987b. Potassium carbonate as a potassium source and dietary buffer for lactating holstein cows during hot weather. J.Dairy Sci. 70:309.

West, J.W., B.G. Mullinix, and T.G. Sandifer. 1990. Changing dietary electrolyte balance for dairy cows in cool and hot environments. J. Dairy Sci. 73:(Suppl. 1):164.(Abstr.)

West, J.W., B.G. Mullinix, and T.G. Sandifer. 1991. The effect of dietary cation-anion balance and cation source on lactational performance and acid-base chemistry of lactating cows during hot, humid weather. J. Dairy Sci. 74:(Suppl. 1):264.

Wheeler, W.E. 1981. Importance of cation-anion balance in ruminant nutrition. Page 17 in Proc. Georgia Nutr. Conf. University of Georgia, Athens.

Williams, C.H., D.J. David, and O. Hsmaa. 1962. The determination of Chromic oxide in faeces samples by atomic absorption spectrophotometry. J. Agric. Sci. 59:381.

Yen, J.T., W.G. Pond, and R.L. Prior. 1981. Calcium chloride as a regulator of feed intake and weight gain in pigs. J. Anim. Sci. 52:778.

BIOGRAPHICAL SKETCH

William Kenneth Sanchez was born in San Diego, California on December 2, 1958. As the seventh child, he learned the importance of proper nutrition and became adept at balancing a diet in a hurry. He was raised in Albuquerque, New Mexico and spent two years in the Sierra Crest Mountains where he became interested in animal agriculture. Oregon was home from 1970 to 1987.

Bill graduated with Bachelor of Science and Master of Science degrees in animal science from Oregon State University. He was active in many professional, community and sports activities while at Oregon State. Bill was nutritionist for Manna Pro Corporation (formerly Carnation Company Milling Division) for three years prior to returning to graduate school. North Florida Holsteins employed Bill as a dairy farm employee prior to his beginning his Ph.D. studies. He currently is graduate research assistant in dairy science at the University of Florida. He is a member of the agricultural honor societies of Gamma Sigma Delta and Alpha Zeta and the American Societies of Animal and Dairy Science.

Bill has accepted a position to teach and conduct research in dairy cattle nutrition at the University of Idaho. Bill and his wife Sandy plan to raise their two children, Melinda and Eric, in Moscow, Idaho, part of the great northwest.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. David K. Beede, Chair
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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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